THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants:	Wang et al.
Appl. No.:	10/598,909
Conf. No.:	1906
Filed:	September 14, 2006
Title:	DELIVERY OF FUNCTIONAL INGREDIENTS
Art Unit:	1655
Examiner:	Qiuwen Mi
Docket No.:	3712036-00753

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANTS' APPEAL BRIEF

Sir:

Appellants submit this Appeal Brief in support of the Notice of Appeal filed on July 11, 2011. This Appeal is taken from the non-final Office Action dated June 29, 2011.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A. by virtue of an Assignment dated August 22, 2008 and recorded at reel 021431, frame 0358 in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellants' legal representative and the Assignee of the above-identified patent application do not know of any prior or pending appeals, interferences or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF CLAIMS

Claims 1-8, 12-14 and 20-28 are pending in the above-identified patent application. Claims 9-11 and 15-19 were previously canceled without disclaimer. Claims 1-8, 12-14 and 2-28 stand rejected. Therefore, Claims 1-8, 12-14 and 2-28 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

IV. STATUS OF AMENDMENTS

A non-final Office Action was mailed on May 5, 2010, in which the Examiner rejected Claim 14 under 35 U.S.C. §112, second paragraph, and Claims 1-8, 12-14 and 20-21 under 35 U.S.C. §103. Appellants filed a Response to the non-final Office Action on August 19, 2009, in which Appellants amended Claim 14 and argued against the rejections. A final Office Action was mailed on November 16, 2010, in which the Examiner withdrew the indefiniteness rejection and rejected Claims 1-8, 12-14 and 20-21 under 35 U.S.C. §103. Appellants filed a Response to the final Office Action on May 11, 2011, in which Appellants amended Claims 1-8, 12-14 and 20-21, newly added Claims 22-28, and argued against the obviousness rejections. The Examiner mailed a non-final Office Action on June 29, 2011, in which the Examiner rejected Claims 1-8, 12-14 and 20-28 under 35 U.S.C. §112, second paragraph, and Claims 1-8, 12-14 and 20-28 under 35 U.S.C. §103. Appellants filed a Notice of Appeal on July 11, 2011. Copies of the non-final Office Action dated May 5, 2010, final Office Action dated November 16, 2010, and non-final Office Action dated June 29, 2011 are included in the Evidence Appendix as Exhibits A, B and C, respectively.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the invention by way of reference to the specification and/or figures for each of the independent claims is provided as follows:

Independent Claim 1 is directed to a miscible primary composition (page 3, lines 1-11) comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof (page 3, lines 1-11), excluding insoluble fibers (page 3, lines 1-11), in a carrier selected from the group consisting of milk, milk protein-containing carriers and combinations thereof (page 5, lines 21-24), wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier (page 3, lines 19-29; page 7, lines 5-12) and the insoluble fibers are removed by centrifuging the carrier after milling (page 3, lines 19-29; page 7, lines 5-12) and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system (page 3, lines 1-11).

Independent Claim 12 is directed to an oral composition comprising a freeze-dried, miscible powder (page 3, lines 1-17) comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof (page 3, lines 1-11), excluding insoluble fibers (page 3, lines 1-11), in a milk or milk protein-containing carrier (page 5, lines 21-24), the oral composition in a form selected from the group consisting of a foodstuff for oral administration, a food supplement, a pet food product, a cosmetic preparation, a pharmaceutical preparation, and combinations thereof (page 3, lines 1-17), wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier (page 3, lines 19-29; page 7, lines 5-12) and the insoluble fibers are removed by centrifuging the carrier after milling (page 3, lines 19-29; page 7, lines 5-12) and wherein the freeze-dried, miscible powder is stable, miscible and dispersible in an aqueous system (page 3, lines 1-11).

Independent Claim 14 is directed to an oral, cosmetic or pharmaceutical composition comprising a miscible primary composition (page 3, lines 1-11) comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting

of whole fruit, vegetable, plant material, and combinations thereof (page 3, lines 1-11), excluding insoluble fibers (page 3, lines 1-11), in a milk or milk protein-containing carrier (page 5, lines 21-24), the oral, cosmetic or pharmaceutical composition being in a form selected from the group consisting of a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement, a wet oral supplement, and combinations thereof (page 8, line 28-page 9, line 4), wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier (page 3, lines 19-29; page 7, lines 5-12) and the insoluble fibers are removed by centrifuging the carrier after milling (page 3, lines 19-29; page 7, lines 5-12) and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system (page 3, lines 1-11).

Independent Claim 22 is directed to a miscible primary composition (page 3, lines 1-11) comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof (page 3, lines 1-11), excluding insoluble fibers (page 3, lines 1-11), in a plant-based milk carrier (page 5, lines 21-24), wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the plant-based milk carrier (page 3, lines 19-29; page 7, lines 5-12) and the insoluble fibers are removed by centrifuging the carrier after milling (page 3, lines 19-29; page 7, lines 5-12) and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system (page 3, lines 1-11).

Although specification citations are given in accordance with C.F.R. 1.192(c), these reference numerals and citations are merely examples of where support may be found in the specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the references numerals and citations below, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology as is done here to comply with rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the

specification. In short, the references numerals and specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1-8, 12-14 and 20-28 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Appellants regard as the invention.

2. Claims 1-8, 14, 20 and 21 are rejected under 35 U.S.C. §103(a) as being unpatentable over JP 09107880 to Osanai ("*Osanai*"), in view of Journal of Agricultural and Food Chemistry to Edenharder et al. ("*Edenharder*"), Eur J. Nutr to Faulks et al. ("*Faulks*") and Royal Society of Chemistry to Hovari et al. ("*Hovari*") and further in view of JP 2003164261 to Imazawa et al. ("*Imazawa*"). Copies of *Osanai*, *Edenharder*, *Faulks*, *Hovari*, and *Imazawa* are included in the Evidence Appendix as Exhibits, D, E, F, G, and H, respectively.

3. Claims 1-8, 12-14 and 20-28 are rejected under 35 U.S.C. §103(a) as being unpatentable over *Osanai*, *Edenharder*, *Faulks*, *Hovari*, and *Imazawa* in view of Korean Patent No. 2003022942 to Hong et al. ("*Hong*"). A copy of *Hong* is included in the Evidence Appendix as Exhibit I.

VII. ARGUMENT

A. LEGAL STANDARDS

1. Definiteness under 35 U.S.C. §112, second paragraph

The standard for determining whether the definitiveness requirement is met under 35 U.S.C. § 112, ¶ 2 is "whether those skilled in the art would understand what is claimed when the claim is read in light of the Specification." *Orthokinetics Inc. v. Safety Travel Chairs Inc,* 1 U.S.P.Q. 2d 1081-1088 (Fed. Cir. 1986). "If the claims, read in light of the Specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the Courts can demand no more." *North American Vaccine Inc. v American Cyanamid Co.,* 28 U.S.P.Q. 2d 1333, 1339 (Fed. Cir. 1993). In this regard, "[p]atent law allows the inventor to be his own lexicographer ... [T]he specification aids in ascertaining the scope and meaning of the language employed in the claims inasmuch as words must be used in the same way in both the claims and the specification. *United States v. Teletronics, Inc.,* 8 U.S.P.Q. 2d 1217, 1220 (Fed. Cir. 1988). By statute, 35 U.S.C. 112, Congress has placed no limitations on how an applicant claims his invention, so long as the specification concludes with claims which particularly point out and distinctly claim that invention." *In re Pilkington,* 162 U.S.P.Q. 145, 148 (C.C.P.A. 1996).

2. Obviousness under 35 U.S.C. §103

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert,* 28 U.S.P.Q. 2d 1955, 1956 (Fed. Cir. 1993). This

burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

Moreover, the Patent Office must provide explicit reasons why the claimed invention is obvious in view of the prior art. The Supreme Court has emphasized that when formulating a rejection under 35 U.S.C. § 103(a) based upon a combination of prior art elements it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed. *KSR v. Teleflex*, 127 S. Ct. 1727 (2007).

Of course, references must be considered as a whole and those portions teaching against or away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered to teach away when a person of ordinary skill, upon reading the reference would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Machinery Corp. v. Fukuhara Industrial Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998), quoting, *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994).

B. THE CLAIMED INVENTION

Independent Claim 1 is directed to a miscible primary composition having at least essential lipophilic and hydrophilic bioactive components of a material selected from whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers. The at least essential lipophilic and hydrophilic bioactive components in a carrier selected from the group cof milk, milk protein-containing carriers and combinations thereof. The essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling. The miscible primary composition is stable, miscible and dispersible in an aqueous system.

Independent Claim 12 is directed to an oral composition having a freeze-dried, miscible powder that includes at least essential lipophilic and hydrophilic bioactive components of a material selected from whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers. The at least essential lipophilic and hydrophilic bioactive components are in a milk or milk protein-containing carrier, and the oral composition is in a form selected from a foodstuff for oral administration, a food supplement, a pet food product, a cosmetic preparation, a pharmaceutical preparation, and combinations thereof. The essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling. The freeze-dried, miscible powder is stable, miscible and dispersible in an aqueous system.

Independent Claim 14 is directed to an oral, cosmetic or pharmaceutical composition having a miscible primary composition including at least essential lipophilic and hydrophilic bioactive components of a material selected from whole fruit, vegetable, plant material, and combinations thereof, excluding insoluble fibers. The at least essential lipophilic and hydrophilic bioactive components are in a milk or milk protein-containing carrier, the oral, cosmetic or pharmaceutical composition being in a form selected a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement, a wet oral supplement, and combinations thereof. The essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling. The miscible primary composition is stable, miscible and dispersible in an aqueous system.

Independent Claim 22 is directed to a miscible primary composition having at least essential lipophilic and hydrophilic bioactive components of a material selected from whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers. The at least essential lipophilic and hydrophilic bioactive components are in a plant-based milk carrier, and the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the plant-based milk carrier. The insoluble fibers are removed by centrifuging the carrier after milling. The miscible primary composition is stable, miscible and dispersible in an aqueous system.

C. CLAIMS 1-8, 12-14 AND 20-28 ARE SUFFICIENTLY DEFINITE TO SATISFY THE REQUIREMENTS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

The standard for determining whether the definiteness requirement is met under 35 U.S.C. §112, second paragraph, is whether those skilled in the art would understand what is claimed when the claim is read in light of the specification. With respect to the presently claimed subject matter, Appellants respectfully disagree with the Examiner's assertion that the term "stable" is unclear. See, non-final Office Action, pages 2-3. Instead, Appellants respectfully submit that the skilled artisan would immediately understand the scope of the claims when read in view of the specification.

In the non-final Office Action, the Examiner states that "it is unclear whether the term 'stable' means without producing precipitation or without growing mold or bacteria? Is it stable at the room temperature or at 50 degree C, or in the refrigerator? Will it precipitate at room temperature for one year? Six month[s] or for one month?" See, non-final Office Action, page 3, lines 2-5. In contrast, Appellants respectfully submit that the skilled artisan would understand that a "stable" food product is a food product that is not particularly reactive in its environment during normal use, and retains its useful properties on the timescale of its expected usefulness. For example, unstable food compositions may undergo phase changes or chemical reactions that can change either or both of the physical and chemical properties of the compositions. Such changes can result in bacterial overgrowth, poor organoleptic properties, rancidification of the food, etc. Indeed, food stability is not a new concept in the art of food products and the meaning of "stability" of a food product would be well known to the skilled artisan.

As such, Appellants respectfully submit that the skilled artisan would understand the term "stable" to mean that the food composition retains is useful properties in its environment during normal use, and for an amount of time of its expected usefulness. Thus, the metes and bounds of the terms "stable" are clear to the skilled artisan in view of the specification, the knowledge of the skilled artisan, as well as commonly used definitions of the terms. Based on at least these noted reasons, Appellants believe that the pending claims fully comply with the requirements of 35 U.S.C. §112, second paragraph.

Accordingly, Appellants respectfully request that the rejection of Claims 1-8, 12-14 and 20-28 under §112, second paragraph, be reconsidered and withdrawn.

D. THE REJECTION OF CLAIMS 1-8, 14, 20 AND 21 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED

1. The Examiner Has Failed to Establish a *prima facie* Case of Obviousness

Appellants respectfully submit that the obviousness rejection of Claims 1-8, 14, 20 and 21 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the non-final Office Action, the Examiner asserts that the combination of *Osanai*, *Edenharder*, *Faulks*, *Hovari*, and *Imazawa* renders the claimed subject matter obvious. See, non-final Office Action, pages 3-7. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose or suggest each and every element of the present claims and the skilled artisan would have no reason to combine the cited references to arrive at the present claims.

a. The Cited References Fail to Disclose or Suggest Each and Every Element of the Present Claims

Independent Claims 1 and 14 recite, in part, that the miscible primary composition is stable, miscible and dispersible in an aqueous system, the composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a milk or milk protein-containing carrier.

Conventional techniques for extracting such bioactive components only extract some of the bioactive components from the fruit or plant material. Water extraction techniques, in which the bioactive components are extracted from insoluble fibers, preserve the natural image and nutritional functions of the bioactive components but are not very efficient. Solvent extraction techniques, while more efficient than water extraction, still fail to extract a substantial portion of the bioactive components from the fruit or plant material and simultaneously impair the nutritional functions of the bioactive components. See, specification, page 1, line 23-page 2, line 28. Therefore, traditional water and solvent extraction techniques are only able to extract a few compounds of the fruit or plant material, leaving some other bioactive materials in the remaining

material. For example, polysaccharides, polyphenols and other non-lipophilic compounds are not extracted together with the lipophilic components such as carotenoids, lipophilic vitamins and other lipids.

The claimed compositions are produced by processes that allow for the extraction of a greater amount of bioactive materials than with traditional water or solvent extraction techniques. The fruit or plant material is mixed in a milk or milk protein-containing medium and separated from insoluble fibers to obtain an aqueous suspension. By using a milk or milk protein-containing carrier to extract the bioactive components from the fruit or plant material and centrifuging the milk or milk protein-containing carrier, the present claims provide compositions having bioactive components with improved miscibility, stability and bioavailability over conventional extraction techniques without the use of organic solvent residues. See, specification, page 3, lines 19-page 4, line 10; and page 7, lines 5-12. By using milk or milk proteins, soy-milk or milk-like proteins from plants, the claimed compositions provide a similar profile of the important nutrients like the whole fruit.

Appellants have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and oil-soluble bioactives of the plant material. Moreover, Appellants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and further provides the claimed composition as a whole to be stable, miscible and dispersible in an aqueous system. See, specification, page 2, lines 22-28 and page 3, lines 6-11.

Osanai, *Edenharder*, *Faulks*, *Hovari* and *Imazawa* fail to disclose or suggest each and every element of independent Claims 1 and 14. *Osanai*, *Edenharder*, *Faulks*, *Hovari* and *Imazawa* alone or in combination fail to disclose or suggest a miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as required, in part, by independent Claims 1 and 14. Instead, *Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. See, *Osanai*, pages 5-6. To distinguish the composition of *Osanai* with that of the claimed compositions,

Appellants previously submitted a Declaration under 37 C.F.R. §1.132 ("*Declaration*") that demonstrates the deficiencies of the prior art with respect to the present claims. A copy of the *Declaration* is attached hereto as Exhibit J.

As supported by the *Declaration*, *Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. Each of the embodiments of the beverage disclosed by *Osanai* at least includes approximately 22.5 grams of lemon. Moreover, lemon is an essential aspect of *Osanai's* beverage as it supplies vitamin C in an amount that is not satisfied with the remaining elements of the beverage. See, *Osanai*, paragraph 12.

As supported by the *Declaration*, an experiment was performed to determine the impact of lemon on cow's milk as taught by *Osanai*. The experiment showed that the addition of 22.5 grams of lemon to 100 ml of milk led to a precipitation/coagulation of a large portion of the milk proteins in the milk causing an obvious lack of miscibility. See, Exhibit A of the *Declaration*. Therefore, upon experimental testing to compare *Osanai's* beverage against the claimed compositions, it is clear that *Osanai* does not provide a miscible primary composition that is stable, miscible and dispersible in an aqueous system.

As supported by the *Declaration*, Appellants have surprisingly found that the milk proteins are essential for the improved extraction of the lipophilic bioactive components according to the claimed invention. The claimed miscible primary compositions comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system provides the optimal conditions for extracting the most lipophilic bioactive components from plant materials. In contrast, because of the precipitation/coagulation of a large portion of the milk proteins in the beverage of *Osanai*, these precipitated or coagulated proteins are immiscible in solution and are no longer free to extract the lipophilic bioactive components of plant materials. This reduces the effectiveness of the extraction and the amount of the extracted bioactive components that could end up in the beverage. As a result, the miscible primary composition of the claimed invention is a distinguishable product over the immiscible beverage resulting from the components and process of *Osanai*.

b. The Skilled Artisan Would Have No Reason to Combine the Cited References to Arrive at the Present Claims

Appellants also respectfully submit that the skilled artisan would have no reason to combine the cited references to arrive at the present claims because the cited references are directed to unrelated products that have completely different objectives. *Osanai* is entirely directed to cow's milk containing vegetables whose main constituent is rapa gourd, wherein the vegetable containing rapa gourd is mixed with cowsmilk. See, *Osanai*, pages 5-6. *Edenharder* is entirely directed to the isolation and characterization of antimutagenic flavonoids from spinach. See, *Edenharder*, Abstract. Indeed, the entire disclosure of *Edenharder* is directed to the purification of antimutagens from spinach by preparative and micropreparative HPLC from a methanol/water extract of dry spinach after removal of lipophilic compounds. *Id.* As such, not only is the subject matter of *Edenharder* nonanalogous art when compared to *Osanai* and the present claims, but *Edenharder* teaches away from the present claims when *Edenharder* discloses removal of lipophilic compounds from the spinach extract.

Similar to *Edenharder*, *Faulks* is entirely directed to the quantification of β-carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modeling of triglyceride-rich lipoprotein plasma fraction. See, *Faulks*, Summary. Like *Edenharder*, the green vegetable of *Faulks* is spinach and the entire disclosure is directed to the kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals. See, *Faulks*, Summary and Introduction. As such, *Faulks* is also nonanalogous art when compared to *Osanai* and the present claims.

Hovari is entirely directed to the effects of flavanoids on human health and the content of flavonoids in specific vegetables. See, *Hovari*, Introduction, Table 1. *Imazawa* is entirely directed to extraction efficiency and preparation of juice in a short time for industrialization. See, *Imazawa*, paragraphs 18 and 19. *Imazawa* discloses processes that include pulverizing coffee beans, fruits, vegetables, etc., adding a dispersing media to the pulverized coffee beans, fruits, vegetables, etc., and then homogenizing the mixture. See, *Imazawa*, Working Examples.

As such, the cited references are clearly directed to unrelated products or processes that have completely different objectives. Moreover, none of the cited references even recognizes the benefits obtained by the presently claimed compositions including, for example, improved bioavailability and miscibility of from extracted fruits or plant materials by milling the material in a milk or milk protein-containing carrier and centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials to remove the insoluble fibers. Such treatments

allow the essential lipophilic and hydrophilic bioactive components to have improved bioavailability and miscibility in the milk or milk protein-containing carrier. See, specification, page 4, lines 1-3.

Finally, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there exists no reason for the skilled artisan to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). In fact, Appellants submit that what the Patent Office has done here is to apply hindsight reasoning by attempting to selectively piece together teachings of each of the references in an attempt to recreate what the claimed invention discloses. Indeed, the skilled artisan must have a reason to combine the cited references to arrive at the present claims. Appellants respectfully submit that such a reason is not present in the instant case.

For at least the reasons discussed above, the cited references fail to disclose or suggest each and every element of independent Claims 1 and 14. Moreover, the cited references fail to even recognize the advantages, unexpected benefits and/or properties of a miscible primary composition that is stable, miscible and dispersible in an aqueous system in accordance with the present claims. As a result, Appellants respectfully submit that independent Claims 1 and 14, along with any claims that depend from Claims 1 and 14, are novel, nonobvious and distinguishable from the cited references.

Accordingly, Appellants respectfully request that the obviousness rejection of Claims 1-8, 14, 20 and 21 under 35 U.S.C. §103 be reconsidered and withdrawn.

2. Even if the Examiner has demonstrated a *prima facie* case of obviousness, Appellants have submitted a *Declaration* that rebuts any *prima facie* case of obviousness

"One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of 'unexpected results,' i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995). Appellants have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and

oil-soluble bioactives of the plant material. Moreover, Appellants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and further provides the claimed composition as a whole to be stable, miscible and dispersible in an aqueous system. See, specification, page 2, lines 22-28 and page 3, lines 6-11.

As supported by the *Declaration*, *Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. Each of the embodiments of the beverage disclosed by *Osanai* at least includes approximately 22.5 grams of lemon. Moreover, lemon is an essential aspect of *Osanai's* beverage as it supplies vitamin C in an amount that is not satisfied with the remaining elements of the beverage. See, *Osanai*, paragraph 12.

As supported by the *Declaration*, an experiment was performed to determine the impact of lemon on cow's milk as taught by *Osanai*. The experiment showed that the addition of 22.5 grams of lemon to 100 ml of milk led to a precipitation/coagulation of a large portion of the milk proteins in the milk causing an obvious lack of miscibility. See, Exhibit A of the *Declaration*. Therefore, upon experimental testing to compare *Osanai's* beverage against the claimed invention, it is clear that *Osanai* does not provide a miscible primary composition that is stable, miscible and dispersible in an aqueous system according to the claimed invention.

As supported by the *Declaration*, Appellants have surprisingly found that the milk proteins are essential for the improved extraction of the lipophilic bioactive components according to the claimed invention. The claimed miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system provides the optimal conditions for extracting the most lipophilic bioactive components from plant materials. In contrast, because of the precipitation/coagulation of a large portion of the milk proteins in the beverage of *Osanai*, these precipitated or coagulated proteins are immiscible in solution and are no longer free to extract the lipophilic bioactive components of plant materials. This reduces the effectiveness of the extraction and the amount of the extracted bioactive components that could end up in the beverage. As a result, the miscible primary composition of the claimed invention is a distinguishable product over the immiscible beverage resulting from the components and process of *Osanai*.

Accordingly, Appellants respectfully request that the obviousness rejection of Claims 1-8, 14, 20 and 21 under 35 U.S.C. §103 be reconsidered and withdrawn.

E. THE REJECTION OF CLAIMS 1-8, 12-14 AND 20-28 UNDER 35 U.S.C. §103(a) SHOULD BE REVERSED

1. The Examiner Has Failed to Establish a *prima facie* Case of Obviousness

Appellants respectfully submit that the obviousness rejection of Claims 1-8, 12-14 and 20-28 should be reversed because the Examiner has failed to establish a *prima facie* case of obviousness. In the non-final Office Action, the Examiner asserts that the combination of *Osanai*, *Edenharder*, *Faulks*, *Hovari*, *Imazawa*, and *Hong* renders the claimed subject matter obvious. See, non-final Office Action, pages 7-9. However, the Examiner has failed to establish a *prima facie* case of obviousness because the cited references fail to disclose or suggest each and every element of the present claims and the skilled artisan would have no reason to combine the cited references to arrive at the present claims.

a. The Cited References Fail to Disclose or Suggest Each and Every Element of the Present Claims

Independent Claims 1, 12 and 14 recite, in part, that the miscible primary composition is stable, miscible and dispersible in an aqueous system. Independent Claim 12 also recites, in part, a freeze-dried, miscible powder comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a milk or milk protein-containing carrier. Independent Claim 22 recites, in part, a miscible primary composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a plant-based milk carrier.

As discussed above, conventional techniques for extracting such bioactive components only extract some of the bioactive components from the fruit or plant material. Water extraction

techniques, in which the bioactive components are extracted from insoluble fibers, preserve the natural image and nutritional functions of the bioactive components but are not very efficient. Solvent extraction techniques, while more efficient than water extraction, still fail to extract a substantial portion of the bioactive components from the fruit or plant material and simultaneously impair the nutritional functions of the bioactive components. See, specification, page 1, line 23-page 2, line 28. Therefore, traditional water and solvent extraction techniques are only able to extract a few compounds of the fruit or plant material, leaving some other bioactive materials in the remaining material. For example, polysaccharides, polyphenols and other non-lipophilic compounds are not extracted together with the lipophilic components such as carotenoids, lipophilic vitamins and other lipids.

The claimed compositions are produced by processes that allow for the extraction of a greater amount of bioactive materials than with traditional water or solvent extraction techniques. The fruit or plant material is mixed in a milk or milk protein-containing medium and separated from insoluble fibers to obtain an aqueous suspension. By using a milk or milk protein-containing carrier to extract the bioactive components from the fruit or plant material and centrifuging the milk or milk protein-containing carrier, the present claims provide compositions having bioactive components with improved miscibility, stability and bioavailability over conventional extraction techniques without the use of organic solvent residues. See, specification, page 3, lines 19-page 4, line 10; page 7, lines 5-12. By using milk or milk proteins, soy-milk or milk-like proteins from plants, the claimed compositions provide a similar profile of the important nutrients like the whole fruit.

As is also discussed above, Appellants have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and oil-soluble bioactives of the plant material. Moreover, Appellants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and further provides the claimed composition as a whole to be stable, miscible and dispersible in an aqueous system. See, specification, page 2, lines 22-28 and page 3, lines 6-11.

Osanai, *Edenharder*, *Faulks*, *Hovari,* and *Imazawa* fail to disclose or suggest each and every element of independent Claims 1, 12, 14, and 22. *Osanai*, *Edenharder*, *Faulks*, *Hovari* and *Imazawa* alone or in combination fail to disclose or suggest a miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as recited, in part, by independent Claims 1, 12, 14 and 22 for at least the reasons set forth above. *Osanai*, *Edenharder*, *Faulks*, *Hovari* and *Imazawa* also fail to a freeze-dried, miscible powder comprising essential lipophilic and hydrophilic bioactive components of a material from whole fruit, vegetable material and/or plant material, excluding insoluble fibers, in a milk or milk protein-containing carrier as recited, in part, by independent Claim 12.

Hong fails to remedy the deficiencies of *Osanai*, *Edenharder*, *Faulks*, *Hovari,* and *Imazawa* because *Hong* also fails to disclose or suggest a miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as recited, in part, by independent Claims 1, 12, 14 and 22. *Hong* also fails to a freeze-dried, miscible powder comprising essential lipophilic and hydrophilic bioactive components of a material from whole fruit, vegetable material and/or plant material, excluding insoluble fibers, in a milk or milk protein-containing carrier as recited, in part, by independent Claim 12.

Although *Hong* discloses a soy oil and rice milk, vegetable-based composition including fermented lactobacillus, the lactobacillus is not an essential lipophilic and hydrophilic bioactive components of a material from whole fruit, vegetable material and/or plant material, excluding insoluble fibers. As such, *Hong* also fails to disclose or suggest the presently claimed miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as recited, in part, by independent Claims 1, 12, 14 and 22.

Additionally, although *Hong* discloses the freeze-drying of a fermented milk to increase the survival rate of a lactobacillus bacteria, such a powdered fermented milk is not a freeze-dried, miscible powder comprising essential lipophilic and hydrophilic bioactive components of a material from whole fruit, vegetable material and/or plant material, excluding insoluble fibers, in a milk or milk protein-containing carrier as recited, in part, by independent Claim 12. As such, *Hong* fails to remedy the deficiencies of *Osanai*, *Edenharder*, *Faulks*, *Hovari,* and *Imazawa*. As such, the cited references fail to disclose or suggest each and every element of the present claims.

b. The Skilled Artisan Would Have No Reason to Combine the Cited References to Arrive at the Present Claims

Appellants also respectfully submit that the skilled artisan would have no reason to combine the cited references to arrive at the present claims because the cited references are directed to unrelated products that have completely different objectives. *Osanai* is entirely directed to cow's milk containing vegetables whose main constituent is rapa gourd, wherein the vegetable containing rapa gourd is mixed with cowsmilk. See, *Osanai*, pages 5-6. *Edenharder* is entirely directed to the isolation and characterization of antimutagenic flavonoids from spinach. See, *Edenharder*, Abstract. Indeed, the entire disclosure of *Edenharder* is directed to the purification of antimutagens from spinach by preparative and micropreparative HPLC from a methanol/water extract of dry spinach after removal of lipophilic compounds. *Id.* As such, not only is the subject matter of *Edenharder* nonanalogous art when compared to *Osanai* and the present claims, but *Edenharder* teaches away from the present claims when *Edenharder* discloses removal of lipophilic compounds from the spinach extract.

Similar to *Edenharder*, *Faulks* is entirely directed to the quantification of β-carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modeling of triglyceride-rich lipoprotein plasma fraction. See, *Faulks*, Summary. Like *Edenharder*, the green vegetable of *Faulks* is spinach and the entire disclosure is directed to the kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals. See, *Faulks*, Summary and Introduction. As such, *Faulks* is also nonanalogous art when compared to *Osanai* and the present claims.

Hovari is entirely directed to the effects of flavanoids on human health and the content of flavonoids in specific vegetables. See, *Hovari*, Introduction, Table 1. *Imazawa* is entirely directed to extraction efficiency and preparation of juice in a short time for industrialization. See, *Imazawa*, paragraphs 18 and 19. *Imazawa* discloses processes that include pulverizing coffee beans, fruits, vegetables, etc., adding a dispersing media to the pulverized coffee beans, fruits, vegetables, etc., and then homogenizing the mixture. See, *Imazawa*, Working Examples. *Hong* is entirely directed to a vegetable-based food product including probiotics in a liquid or powdery fermented milk form. The purpose of providing such a vegetable-based is to avoid

adverse reactions after consumption by individuals that are lactose intolerant. See, *Hong*, translation, page 2, "Purpose of the Invention."

As such, the cited references are clearly directed to unrelated products or processes that have completely different objectives. Moreover, none of the cited references even recognizes the benefits obtained by the presently claimed compositions including, for example, improved bioavailability and miscibility of from extracted fruits or plant materials by milling the material in a milk or milk protein-containing carrier and centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials to remove the insoluble fibers. Such treatments allow the essential lipophilic and hydrophilic bioactive components to have improved bioavailability and miscibility in the milk or milk protein-containing carrier. See, specification, page 4, lines 1-3.

Finally, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there exists no reason for the skilled artisan to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). In fact, Appellants submit that what the Patent Office has done here is to apply hindsight reasoning by attempting to selectively piece together teachings of each of the references in an attempt to recreate what the claimed invention discloses. Indeed, the skilled artisan must have a reason to combine the cited references to arrive at the present claims. Appellants respectfully submit that such a reason is not present in the instant case.

For at least the reasons discussed above, the cited references fail to disclose or suggest each and every element of independent Claims 1, 12, 14 and 22. Moreover, the cited references fail to even recognize the advantages, unexpected benefits and/or properties of a miscible primary composition that is stable, miscible and dispersible in an aqueous system in accordance with the present claims. As a result, Appellants respectfully submit that independent Claims 1, 12, 14 and 22, along with any claims that depend from Claims 1, 12, 14, and 22 are novel, nonobvious and distinguishable from the cited references.

Accordingly, Appellants respectfully request that the obviousness rejection of Claims 1-8, 12-14 and 20-28 under 35 U.S.C. §103(a) be reconsidered and withdrawn.

2. Even if the Examiner has demonstrated a *prima facie* case of obviousness, Appellants have submitted a *Declaration* that rebuts any *prima facie* case of obviousness

"One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of 'unexpected results,' i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995). Appellants have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and oil-soluble bioactives of the plant material. Moreover, Appellants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and further provides the claimed composition as a whole to be stable, miscible and dispersible in an aqueous system. See, specification, page 2, lines 22-28 and page 3, lines 6-11.

As discussed above, Appellants previously submitted a *Declaration* that demonstrates the deficiencies of the prior art with respect to the present claims. As supported by the *Declaration*, *Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. Each of the embodiments of the beverage disclosed by *Osanai* at least includes approximately 22.5 grams of lemon. Moreover, lemon is an essential aspect of *Osanai's* beverage as it supplies vitamin C in an amount that is not satisfied with the remaining elements of the beverage. See, *Osanai*, paragraph 12.

As supported by the *Declaration*, an experiment was performed to determine the impact of lemon on cow's milk as taught by *Osanai*. The experiment showed that the addition of 22.5 grams of lemon to 100 ml of milk led to a precipitation/coagulation of a large portion of the milk proteins in the milk causing an obvious lack of miscibility. See, Exhibit A of the *Declaration*. Therefore, upon experimental testing to compare *Osanai's* beverage against the claimed invention, it is clear that *Osanai* does not provide a miscible primary composition that is stable, miscible and dispersible in an aqueous system according to the claimed compositions.

As supported by the *Declaration*, Appellants have surprisingly found that the milk proteins are essential for the improved extraction of the lipophilic bioactive components

according to the claimed invention. The claimed miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system provides the optimal conditions for extracting the most lipophilic bioactive components from plant materials. In contrast, because of the precipitation/coagulation of a large portion of the milk proteins in the beverage of *Osanai*, these precipitated or coagulated proteins are immiscible in solution and are no longer free to extract the lipophilic bioactive components of plant materials. This reduces the effectiveness of the extraction and the amount of the extracted bioactive components that could end up in the beverage. As a result, the miscible primary composition of the claimed invention is a distinguishable product over the immiscible beverage resulting from the components and process of *Osanai*.

Accordingly, Appellants respectfully request that the obviousness rejection of Claims 1-8, 12-14 and 20-28 under 35 U.S.C. §103 be reconsidered and withdrawn.

VIII. CONCLUSION

Appellants respectfully submit that Claims 1-8, 12-14 and 20-28 meet the requirements of 35 U.S.C. §112, second paragraph. Appellants further submit that the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103 and that, even if the Examiner has established a *prima facie* case of obviousness, Appellants have rebutted any showing of obviousness by demonstrating unexpected results. Accordingly, Appellants respectfully submit that the indefiniteness and obviousness rejections are erroneous in law and in fact and should, therefore, be reversed by this Board.

The Director is authorized to charge $540 for the Appeal Brief and any additional fees which may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 3712036-00753 on the account statement.

Respectfully submitted,

K&L GATES LLP

BY _____

Robert M. Barrett
Reg. No. 30,142
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Dated: September 2, 2011

CLAIMS APPENDIX

PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 10/598,909

1. A miscible primary composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a carrier selected from the group consisting of milk, milk protein-containing carriers and combinations thereof, wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system.

2. The miscible primary composition according to claim 1, wherein the material is in a form selected from the group consisting of vegetables, leaves, flowers, fruits, seeds and other parts of the plant, and combinations thereof.

3. The miscible primary composition according to claim 1, wherein the material is selected from the group consisting of a berry, vegetables, seeds, flowers, citrus fruits, tomato, spinach, celery, carrots, pea, kale, parsley, watercress, cabbage, broccoli, lettuce, brussels sprouts, collard greens, turnip greens, fennel, onions, tea, corn, cocoa, coffee, thyme, sweet red pepper, and combinations thereof.

4. The miscible primary composition according to claim 1, wherein the essential lipophilic and hydrophilic bioactive components are selected from the group consisting of lipids, alkaloids, proteins, carbohydrates, carotenoids, polyphenolic compounds, vitamins, minerals, and combinations thereof.

5. The miscible primary composition according to claim 1, wherein the essential lipophilic and hydrophilic bioactive components are flavonoids selected from the group consisting of flavones, flavonols, flavanones, catechins, anthocyanidins, isoflavones, and combinations thereof.

6. The miscible primary composition according to claim 4, wherein the carotenoids are selected from the group consisting of carotenes, xanthophylls, and combinations thereof.

7. The miscible primary composition according to claim 1, wherein the milk is from animal origin.

8. The miscible primary composition according to claim 1, which is in a form selected from the group consisting of a powder, gel, liquid, and combinations thereof.

12. An oral composition comprising a freeze-dried, miscible powder comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a milk or milk protein-containing carrier, the oral composition in a form selected from the group consisting of a foodstuff for oral administration, a food supplement, a pet food product, a cosmetic preparation, a pharmaceutical preparation, and combinations thereof, wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling and wherein the freeze-dried, miscible powder is stable, miscible and dispersible in an aqueous system.

13. The oral composition according to claim 12, which is a form selected from the group consisting of a nutritional complete formula, a dairy product, a chilled or shelf stable beverage, a mineral or purified water, a liquid drink, a soup, a dietary supplement, a meal replacement, a nutritional bar, a confectionery, a milk or a fermented milk product, a yoghurt, a milk based powder, an enteral nutrition product, an infant formula, an infant nutritional product, a cereal product or a fermented cereal based product, an ice-cream, a chocolate, coffee, a culinary product , salad dressings, a pet food, and combinations thereof.

14. An oral, cosmetic or pharmaceutical composition comprising a miscible primary composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable, plant material, and combinations thereof, excluding insoluble fibers, in a milk or milk protein-containing carrier, the oral, cosmetic or pharmaceutical composition being in a form selected from the group consisting of a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement, a wet oral supplement, and combinations thereof, wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system.

20. The miscible primary composition according to claim 1, wherein the material is selected from the group consisting of wolfberry, blueberry, cranberry, white currant, red currant, blackcurrant, mulberry, blackberry, gooseberry, raspberry, sea buckthorn, strawberry, arbutus berry, grapes, flavonoid, polyphenol carotenoid-rich fruit, vegetables, seeds, flowers, apples, melons, kiwi, cherries, red date, prunes, peaches, persimmons, mandarin, orange, tangerine, grapefruit, chamomile, chrysanthemum, bitter orange, honeysuckle, jasmine and safflower, tomato, spinach, celery, carrots, pea, kale, parsley, watercress, cabbage, broccoli, lettuce, brussels sprouts, collard greens, turnip greens, fennel, onions, tea, corn, cocoa, coffee, thyme, sweet red pepper, and combinations thereof.

21. The miscible primary composition according to claim 1, wherein the essential lipophilic and hydrophilic bioactive components are selected from the group consisting of apigenin, luteolin, diosmetin, quercetin, myricetin, kaempferol, naringenin, hesperidin, epicatechin, gallocatechin, pelargonidin, malvidin, cyaniding, genistein, daidzein, and combinations thereof.

22. A miscible primary composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material, and combinations thereof, excluding insoluble fibers, in a plant-based milk carrier, wherein the essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the plant-based milk carrier and the insoluble fibers are removed by centrifuging the carrier after milling and wherein the miscible primary composition is stable, miscible and dispersible in an aqueous system.

23. The miscible primary composition according to claim 22, wherein the material is in a form selected from the group consisting of vegetables, leaves, flowers, fruits, seeds and other parts of the plant, and combinations thereof.

24. The miscible primary composition according to claim 22, wherein the material is selected from the group consisting of a berry, vegetables, seeds, flowers, citrus fruits, tomato, spinach, celery, carrots, pea, kale, parsley, watercress, cabbage, broccoli, lettuce, brussels sprouts, collard greens, turnip greens, fennel, onions, tea, corn, cocoa, coffee, thyme, sweet red pepper, and combinations thereof.

25. The miscible primary composition according to claim 22, wherein the plant-based milk carrier is selected from the group consisting of soymilk, coconut milk and combinations thereof.

26. The miscible primary composition according to claim 22, wherein the essential lipophilic and hydrophilic bioactive components are flavonoids selected from the group consisting of flavones, flavonols, flavanones, catechins, anthocyanidins, isoflavones, and combinations thereof.

27. The miscible primary composition according to claim 26, wherein the carotenoids are selected from the group consisting of carotenes, xanthophylls, and combinations thereof.

28. The miscible primary composition according to claim 22, which is in a form selected from the group consisting of a powder, gel, liquid, and combinations thereof.

EVIDENCE APPENDIX

EXHIBIT A: Non-final Office Action dated May 5, 2010

EXHIBIT B: Final Office Action dated November 16, 2010

EXHIBIT C: Non-final Office Action dated June 29, 2011

EXHIBIT D: JP 09107880 to Osanai ("*Osanai*"),

EXHIBIT E: Journal of Agricultural and Food Chemistry to Edenharder et al. ("*Edenharder*")

EXHIBIT F: Eur J. Nutr to Faulks et al. ("*Faulks*")

EXHIBIT G: Royal Society of Chemistry to Hovari et al. ("*Hovari*")

EXHIBIT H: JP 2003164261 to Imazawa et al. ("*Imazawa*")

EXHIBIT I: Korean Patent No. 2003022942 to Hong et al. ("*Hong*")

EXHIBIT J: *Declaration* of Junkuan Wang under 37 C.F.R. §1.132

RELATED PROCEEDINGS APPENDIX

None.

EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/598,909	09/14/2006	Junkuan Wang	3712036.00753	1906

29157 7590 05/05/2010

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EXAMINER	
MI, QIUWEN	
ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
05/05/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

Office Action Summary	Application No.	Applicant(s)	
	10/598,909	WANG ET AL.	
	Examiner	Art Unit	
	QIUWEN MI	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 April 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-21* is/are pending in the application.

4a) Of the above claim(s) *9-11 and 15-19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8, 12-14, 20, and 21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on 4/14/2010 is acknowledged. Claims 1-21 are pending. Claims 9-11, and 15-19 are withdrawn as they are directed toward non-elected invention groups. **Claims 1-8, 12-14, 20, and 21 are examined on the merits**.

Any rejection that is not reiterated is hereby withdrawn.

Claim Rejections –35 USC § 112, 2nd

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 14 is newly rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 14 recites "…which is in a tablet form selected from the group consisting of a capsule, a pill, a solution, a suspension, a syrup, a dried oral supplement,, a wet oral supplement, and combinations thereof..." (lines 5-7). It is not clear how a tablet form could be selected from a capsule, a solution, a suspension, or a syrup. In addition, it is uncertain, what "which" refers to here, an oral composition or the carrier.

Therefore, the metes and bounds of claims are rendered vague and indefinite. The lack of clarity renders the claims very confusing and ambiguous since the resulting claims do not clearly set forth the metes and bounds of the patent protection desired.

Claim Rejections –35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-8, 12-14, 20, and 21 are newly rejected under 35 U.S.C. 103(a) as being unpatentable over Osanai (JP 09107880 A), in view of Edenharder et al (Edenharder et al, Isolation and characterization of structurally novel antimutagenic flavonoids from spinach (Spinacia oleracea), Journal of agricultural and food chemistry, (2001 Jun) Vol. 49, No. 6, pp. 2767-73), Faulks et al (Faulks et al, Kinetic of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals, Eur J Nutr (2004) 43: 15-22), and Hovari et al (Hovari et al, Examination of flavonoid content in Hungarian Vegetables, Special Publication - Royal Society of Chemistry (1999), 240(Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease), 296-298), and further in view of Imazawa et al (JP 2003164261 A).

This is a new rejection necessitated by the Applicant's amendment filed on 4/14/2010.

Osanai teaches to produce a suitably producible cow's milk (thus milk from animal origin, thus a carrier) at a low cost by using a widely used vegetable, capable of enriching iron, enhancing hematopoietic actions, further containing various vitamins or minerals blended in

good balance and effective against various symptoms of anemia, constipation or climacteric disturbance of women (thus a food, thus an oral composition). This cow's milk contains a vegetable and is obtained by adding about 12.5 g KOMATSU-NA [Brassica campestris (rapa groupl), about 2.5 g spinach (thus a vegetable, thus a leave), about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant, 22.5 g lemon (thus a fruit) and 2.5 g reducing palatinose with about 150cc cow's milk. Furthermore, the cow's milk containing the vegetable is prepared by placing about 12.5 g KOMATSU-NA, about 2.5 g spinach and about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10 cc cow's milk in a mixer, pulverizing (thus milling in milk) and mixing the ingredients, adding about 22.5 g lemon and about 2.5 g reducing palatinose thereto and further adding cow's milk thereto so as to make the sum total to 200 cc (thus a liquid, thus a miscible primary composition) (see Abstract). Osanai teaches a method of producing cowsmilk containing vegetables characterized as placing approximately 15 g of carrots, approximately 22.2 g of lemon, and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing it and mixing it, straining it in a strainer twice (thus excluding insoluble fibers), and then adding cowsmilk to this so that it reaches 200 cc (page 5, claim 6 of the full translation).

As evidenced by Edenharder et al, spinach contains carotenoids (thus a hydrophilic bioactive component) and flavonoids such as flavonol and flavanone (thus a lipophilic bioactive component) (see Abstract), therefore, the milk product of Osanai that contains spinach contains at least essential lipophilic and hydrophilic bioactive components consisting of vegetable etc.

As further evidenced by Faulks et al, spinach contains beta-carotene (thus a hydrophilic bioactive component) (see Abstract).

As also evidenced by Hovari et al, the highest quercetin concentration could be detected in different types of onion (67.1-171.3 mg/kg) and in spinach (page 296, last paragraph) (thus the limitation of claim 21 is met).

Osanai does not teach the insoluble fibers are removed by centrifuging the carrier after milling.

Imazawa et al teach a method for manufacturing extract and/or squeezed liquid, involves grinding raw material, homogenizing, dispersing in medium at less than 60 degrees C, extracting, emulsifying and removing dregs and/or squeezed dregs. The raw materials are selected from coffee, green tea (thus containing lipophilic and hydrophilic bioactive components), black tea, oolong tea, herb tea, wild grass tea, chinese medicine tea , cocoa, vanilla, fruits or vegetables. The dispersion medium has low temperature of less than 50 degrees C preferably -5-50 degrees C. The dispersion medium is water, cow's milk (thus a carrier) dairy products, liquid of saccharides, sugar alcohol, mineral, vitamin, stabilizer, emulsifier and bacteriostatic. The mixture is homogenized using homogenous machine (thus milling the material) equipped with pump, which pours dispersion liquid at high voltage and high speed continuously in the homogenous valve (see Abstract). Imazawa et al also teach in accordance with a conventional method, separation removal of extraction slag and/or the juice slag is carried out using a liquid cyclone, a clarifier, centrifugal separation (thus insoluble fibers are removed by centrifuging the carrier after milling), filatration, precision filtration, decantation etc [0027] (see machine translation attached). Imazawa et al teach the method is suitable for the continuous mass production and extremely effective from the viewpoint of the effective utilization of food resources and the economic merit compared with conventional extraction/squeezing method (see Abstract).

First of all, the MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see MPEP 2113 [R-1]). Therefore, although Osanai teaches using strainers twice, instead of using claimed centrifuging process, insoluble fibers are being removed either way, and the final products are not materially different. Even if there is subtle difference between using strainers and centrifuge machine, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use the claimed centrifuging step since Imazawa et al teach removing extraction slag by a liquid cyclone, a clarifier, centrifugal separation, filatration, precision filtration, or decantation. It is evidenced by Imazawa et al that centrifuging step is well known in the art to remove extraction slags, and it is used interchangeably in the art with other methods such as filtration or straining. Since Imazawa et al teach using dispersion medium cowsmilk to grind raw plant material for extraction, and since Imazawa et al teach the method is extremely effective in utilization of food resources and has economic merit compared with conventional extraction/squeezing method, one of the ordinary skills in the art would have been motivated to combine the teachings of the references together.

From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Qiuwen Mi whose telephone number is 571-272-5984. The examiner can normally be reached on 8 to 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on 571-272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Qiuwen Mi/

Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 10/598,909	Applicant(s)/Patent Under Reexamination WANG ET AL.	
	Examiner QIUWEN MI	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	JP 2003164261 A	06-2003	Japan	IMAZAWA et al.	----------
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT B

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/598,909	09/14/2006	Junkuan Wang	3712036.00753	1906

29157 7590 11/16/2010

K&L Gates LLP
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EXAMINER	
MI, QIUWEN	

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
11/16/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

Office Action Summary	Application No.	Applicant(s)	
	10/598,909	WANG ET AL.	
	Examiner	Art Unit	
	QIUWEN MI	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 November 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-21* is/are pending in the application.

4a) Of the above claim(s) *9-11 and 15-19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,12-14,20 and 21* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on 11/5/2010 is acknowledged. Claims 1-21 are pending. Claims 9-11, and 15-19 are withdrawn as they are directed toward non-elected invention groups. **Claims 1-8, 12-14, 20, and 21 are examined on the merits**.

Any rejection that is not reiterated is hereby withdrawn.

Claim Rejections –35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-8, 12-14, 20, and 21 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Osanai (JP 09107880 A), in view of Edenharder et al (Edenharder et al, Isolation and characterization of structurally novel antimutagenic flavonoids from spinach (Spinacia oleracea), Journal of agricultural and food chemistry, (2001 Jun) Vol. 49, No. 6, pp. 2767-73), Faulks et al (Faulks et al, Kinetic of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals, Eur J Nutr (2004) 43: 15-22), and Hovari et al (Hovari et al, Examination of flavonoid content in Hungarian Vegetables, Special Publication - Royal Society of Chemistry (1999), 240(Natural Antioxidants and Anticarcinogens

in Nutrition, Health and Disease), 296-298), and further in view of Imazawa et al (JP 2003164261 A).

This rejection is maintained for reasons of record set forth in the Office Action mailed out on 5/5/2010, repeated below. Applicants' arguments filed have been fully considered but they are not deemed to be persuasive.

Osanai teaches to produce a suitably producible cow's milk (thus milk from animal origin, thus a carrier) at a low cost by using a widely used vegetable, capable of enriching iron, enhancing hematopoietic actions, further containing various vitamins or minerals blended in good balance and effective against various symptoms of anemia, constipation or climacteric disturbance of women (thus a food, thus an oral composition). This cow's milk contains a vegetable and is obtained by adding about 12.5 g KOMATSU-NA [Brassica campestris (rapa groupl), about 2.5 g spinach (thus a vegetable, thus a leave), about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant, 22.5 g lemon (thus a fruit) and 2.5 g reducing palatinose with about 150cc cow's milk. Furthermore, the cow's milk containing the vegetable is prepared by placing about 12.5 g KOMATSU-NA, about 2.5 g spinach and about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10 cc cow's milk in a mixer, pulverizing (thus milling in milk) and mixing the ingredients, adding about 22.5 g lemon and about 2.5 g reducing palatinose thereto and further adding cow's milk thereto so as to make the sum total to 200 cc (thus a liquid, thus a miscible primary composition) (see Abstract). Osanai teaches a method of producing cowsmilk containing vegetables characterized as placing approximately 15 g of carrots, approximately 22.2 g of lemon, and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing it and mixing it, straining it

in a strainer twice (thus excluding insoluble fibers), and then adding cowsmilk to this so that it reaches 200 cc (page 5, claim 6 of the full translation).

As evidenced by Edenharder et al, spinach contains carotenoids (thus a hydrophilic bioactive component) and flavonoids such as flavonol and flavanone (thus a lipophilic bioactive component) (see Abstract), therefore, the milk product of Osanai that contains spinach contains at least essential lipophilic and hydrophilic bioactive components consisting of vegetable etc.

As further evidenced by Faulks et al, spinach contains beta-carotene (thus a hydrophilic bioactive component) (see Abstract).

As also evidenced by Hovari et al, the highest quercetin concentration could be detected in different types of onion (67.1-171.3 mg/kg) and in spinach (page 296, last paragraph) (thus the limitation of claim 21 is met).

Osanai does not teach the insoluble fibers are removed by centrifuging the carrier after milling.

Imazawa et al teach a method for manufacturing extract and/or squeezed liquid, involves grinding raw material, homogenizing, dispersing in medium at less than 60 degrees C, extracting, emulsifying and removing dregs and/or squeezed dregs. The raw materials are selected from coffee, green tea (thus containing lipophilic and hydrophilic bioactive components), black tea, oolong tea, herb tea, wild grass tea, chinese medicine tea , cocoa, vanilla, fruits or vegetables. The dispersion medium has low temperature of less than 50 degrees C preferably -5-50 degrees C. The dispersion medium is water, cow's milk (thus a carrier) dairy products, liquid of saccharides, sugar alcohol, mineral, vitamin, stabilizer, emulsifier and bacteriostatic. The mixture is homogenized using homogenous machine (thus milling the material) equipped with pump,

which pours dispersion liquid at high voltage and high speed continuously in the homogenous valve (see Abstract). Imazawa et al also teach in accordance with a conventional method, separation removal of extraction slag and/or the juice slag is carried out using a liquid cyclone, a clarifier, centrifugal separation (thus insoluble fibers are removed by centrifuging the carrier after milling), filatration, precision filtration, decantation etc [0027] (see machine translation attached). Imazawa et al teach the method is suitable for the continuous mass production and extremely effective from the viewpoint of the effective utilization of food resources and the economic merit compared with conventional extraction/squeezing method (see Abstract).

First of all, the MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not directly added, but is instead produced in-situ does not change the end product" (see MPEP 2113 [R-1]). Therefore, although Osanai teaches using strainers twice, instead of using claimed centrifuging process, insoluble fibers are being removed either way, and the final products are not materially different. Even if there is subtle difference between using strainers and centrifuge machine, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use the claimed centrifuging step since Imazawa et al teach removing extraction slag by a liquid cyclone, a clarifier, centrifugal separation, filatration, precision

filtration, or decantation. It is evidenced by Imazawa et al that centrifuging step is well known in the art to remove extraction slags, and it is used interchangeably in the art with other methods such as filtration or straining. Since Imazawa et al teach using dispersion medium cowsmilk to grind raw plant material for extraction, and since Imazawa et al teach the method is extremely effective in utilization of food resources and has economic merit compared with conventional extraction/squeezing method, one of the ordinary skills in the art would have been motivated to combine the teachings of the references together.

From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Applicant argues that "*Osanai* is entirely directed to cow's milk containing vegetables whose main constituent is rapa gourd, wherein the vegetable containing rapa gourd is mixed with cowsmilk. See, *Osanai,* pages 5-6. *Edenharder* is entirely directed to the isolation and characterization of antimutagenic flavonoids from spinach. See, *Edenharder,* Abstract. Indeed, the entire disclosure of *Edenharder* is directed to the purification of antimutagens from spinach by preparative and micropreparative HPLC from a methanol/water extract of dry spinach after removal of lipophilic compounds. See, *Id.* As such, not only is the subject matter of *Edenharder* nonanalogous art when compared to *Osanai* and the present claims, but *Edenharder* teaches away from the present claims when *Edenharder* discloses removal of lipophilic compounds from the spinach extract. Similar to *Edenharder, Faulks* is entirely directed to the quantification of [3-

carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modeling of triglyceride-rich lipoprotein plasma fraction. See, *Faulks,* Summary. Like *Edenharder,* the green vegetable *of Faulks* is spinach and the entire disclosure is directed to the kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals. See, *Faulks,* Summary and Introduction. As such, *Faulks* is also nonanalogous art when compared to *Osanai* and the present claims. *Hovari* is entirely directed to the effects of flavanoids on human health and the content of flavonoids in specific vegetables. See, *Hovari,* Introduction, Table 1. *lmazawa* is entirely directed to extraction efficiency and preparation of juice in a short time for industrialization. See, *lmazawa,* paragraphs 18 and 19. *Imazawa* is entirely directed to processes that include pulverizing the coffee beans, fruits, vegetables, etc., adding a dispersing media to the pulverized coffee beans, fruits, vegetables, etc., and then homogenizing the mixture. See, *lmazawa,* Working Examples. As such, the cited references are clearly directed to unrelated products or processes that have completely different objectives. Moreover, none of the cited references even recognizes the benefits obtained from the presently claimed compositions including, for example, improved bioavailability and miscibility from extracted fruits or plant materials by milling the material in a milk or milk protein-containing carrier and centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials to remove the insoluble fibers. Such treatments allow the essential lipophilic and hydrophilic bioactive components to have improved bioavailability and miscibility in the milk or milk protein-containing cartier. See, specification, page 4, lines 1-3" (page 9, 2nd paragraph to page 10, 2nd paragraph).

This is not found persuasive. The rejection is based on Osanai in view of Imazaawa, references Edenharder et al, Faulks et al, and Hovari et al are only brought in to show the intrinsic properties of the product in Osanai. Osanai teaches "the cow's milk containing the vegetable is prepared by placing about 12.5 g KOMATSU-NA, about 2.5 g spinach and about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10 cc cow's milk in a mixer, pulverizing (thus milling in milk) and mixing the ingredients" (see Abstract). The process of mixing the claimed ingredient with milk in a mixer, pulverizing, and mixing the ingredient is not materially different from the claimed "milling the material in the milk or milk protein-containing carrier". Although Osanai teaches using strainers twice, instead of using claimed centrifuging process, insoluble fibers are being removed either way, and the final products are not materially different. Even if there is subtle difference between using strainers and centrifuge machine, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use the claimed centrifuging step since Imazawa et al teach removing extraction slag by a liquid cyclone, a clarifier, centrifugal separation, filatration, precision filtration, or decantation. It is evidenced by Imazawa et al that centrifuging step is well known in the art to remove extraction slags, and it is used interchangeably in the art with other methods such as filtration or straining. Since Imazawa et al teach using dispersion medium cowsmilk to grind raw plant material for extraction, and since Imazawa et al teach the method is extremely effective in utilization of food resources and has economic merit compared with conventional extraction/squeezing method, one of the ordinary skills in the art would have been motivated to combine the teachings of the references together.

Applicant argues "Further, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there exists no reason for the skilled artisan to make the proposed modification. *In re Gordon,* 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). In fact, Applicants submit that what the Patent Office has done here is to apply hindsight reasoning by attempting to selectively piece together teachings of each of the references in an attempt to recreate what the claimed invention discloses. Indeed, the skilled artisan must have a reason to combine the cited references to arrive at the present claims. Applicants respectfully submit that such a reason is not present in the instant case" (page 10, 3rd paragraph).

In response to Appellant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the Appellant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Applicant's arguments have been fully considered but they are not persuasive, and therefore the rejections in the record are maintained.

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Qiuwen Mi whose telephone number is 571-272-5984. The examiner can normally be reached on 8 to 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on 571-272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Qiuwen Mi/

Primary Examiner, Art Unit 1655

EXHIBIT C

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/598,909	09/14/2006	Junkuan Wang	3712036.00753	1906

29157 7590 06/29/2011

K&L Gates LLP
P.O. Box 1135
CHICAGO, IL 60690

EXAMINER	
MI, QIUWEN	

ART UNIT	PAPER NUMBER
1655	

NOTIFICATION DATE	DELIVERY MODE
06/29/2011	ELECTRONIC

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Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

chicago.patents@klgates.com

Office Action Summary	Application No.	Applicant(s)	
	10/598,909	WANG ET AL.	
	Examiner	Art Unit	
	QIUWEN MI	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 May 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,12-14 and 20-28* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,12-14 and 20-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

CONTINUED EXAMINATIONS

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/11/2011 has been entered.

Applicant's amendment and 132 Declaration filed on 5/11/2011 are acknowledged, with the cancellation of claims 9-11, 15-19, and newly added claims 22-28. Claims 1-8, 12-14, and 20-28 are pending. **Claims 1-8, 12-14, and 20-28 are examined on the merits**.

Any rejection that is not reiterated is hereby withdrawn.

Claim Rejections –35 USC § 112, 2nd

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-8, 12-14, and 20-28 are newly rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 (at line 9), Claims 12 and 14 (at line 11), Claim 22 (at line 7) recite "stable". The term "stable" in claims 1, 12, 14, and 22 is a relative term which renders the claim indefinite. The term "stable" is not defined by the claim, the specification does not provide a standard for

ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. For instance, it is unclear whether the term "stable" means without producing precipitation or without growing mold or bacterial? Is it stable at the room temperature or at 50 degree C, or in the refrigerator? Will it precipitate at room temperature for one year? Six month or for one month?

Therefore, the metes and bounds of claims are rendered vague and indefinite. The lack of clarity renders the claims very confusing and ambiguous since the resulting claims do not clearly set forth the metes and bounds of the patent protection desired.

All other cited claims depend directly or indirectly from rejected claims and are, therefore, also, rejected under U.S.C. 112, second paragraph for the reasons set forth above.

Claim Rejections –35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-8, 14, 20, and 21 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Osanai (JP 09107880 A), in view of Edenharder et al (Edenharder et al, Isolation and characterization of structurally novel antimutagenic flavonoids from spinach (Spinacia oleracea), Journal of agricultural and food chemistry, (2001 Jun) Vol. 49, No. 6, pp. 2767-73), Faulks et al

(Faulks et al, Kinetic of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals, Eur J Nutr (2004) 43: 15-22), and Hovari et al (Hovari et al, Examination of flavonoid content in Hungarian Vegetables, Special Publication - Royal Society of Chemistry (1999), 240(Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease), 296-298), and further in view of Imazawa et al (JP 2003164261 A).

This rejection is maintained for reasons of record set forth in the Office Action mailed out on 11/16/2010, repeated below, slightly altered to take into consideration Applicant's amendment filed on 5/11/2011. Applicants' arguments filed have been fully considered but they are not deemed to be persuasive.

Osanai teaches to produce a suitably producible cow's milk (thus milk from animal origin, thus a carrier) at a low cost by using a widely used vegetable, capable of enriching iron, enhancing hematopoietic actions, further containing various vitamins or minerals blended in good balance and effective against various symptoms of anemia, constipation or climacteric disturbance of women (thus a food, thus an oral composition). This cow's milk contains a vegetable and is obtained by adding about 12.5 g KOMATSU-NA [Brassica campestris (rapa groupl), about 2.5 g spinach (thus a vegetable, thus a leave), about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant, 22.5 g lemon (thus a fruit) and 2.5 g reducing palatinose with about 150cc cow's milk. Furthermore, the cow's milk containing the vegetable is prepared by placing about 12.5 g KOMATSU-NA, about 2.5 g spinach and about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10 cc cow's milk in a mixer, pulverizing (thus milling in milk) and mixing the ingredients, adding about 22.5 g lemon and about 2.5 g reducing palatinose thereto and further adding cow's milk thereto so as

to make the sum total to 200 cc (thus a liquid, thus a miscible, and dispersible primary composition) (see Abstract). Osanai teaches a method of producing cowsmilk containing vegetables characterized as placing approximately 15 g of carrots, approximately 22.2 g of lemon, and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing it and mixing it, straining it in a strainer twice (thus excluding insoluble fibers), and then adding cowsmilk to this so that it reaches 200 cc (page 5, claim 6 of the full translation). Osanai also teaches Table 1 indicated the comparative examples. A regulated soymilk is commonly known as "regulated soymilk" (thus a plant-based milk carrier) from company A wherein the soymilk has been regulated (page 18, [0014]). Osanai further teaches

As evidenced by Edenharder et al, spinach contains carotenoids (thus a hydrophilic bioactive component) and flavonoids such as flavonol and flavanone (thus a lipophilic bioactive component) (see Abstract), therefore, the milk product of Osanai that contains spinach contains at least essential lipophilic and hydrophilic bioactive components consisting of vegetable etc.

As further evidenced by Faulks et al, spinach contains beta-carotene (thus a hydrophilic bioactive component) (see Abstract).

As also evidenced by Hovari et al, the highest quercetin concentration could be detected in different types of onion (67.1-171.3 mg/kg) and in spinach (page 296, last paragraph) (thus the limitation of claim 21 is met).

Osanai does not teach the insoluble fibers are removed by centrifuging the carrier after milling.

Imazawa et al teach a method for manufacturing extract and/or squeezed liquid, involves grinding raw material, homogenizing, dispersing in medium at less than 60 degrees C, extracting,

emulsifying and removing dregs and/or squeezed dregs. The raw materials are selected from coffee, green tea (thus containing lipophilic and hydrophilic bioactive components), black tea, oolong tea, herb tea, wild grass tea, chinese medicine tea , cocoa, vanilla, fruits or vegetables. The dispersion medium has low temperature of less than 50 degrees C preferably -5-50 degrees C. The dispersion medium is water, cow's milk (thus a carrier) dairy products, liquid of saccharides, sugar alcohol, mineral, vitamin, stabilizer, emulsifier and bacteriostatic. The mixture is homogenized using homogenous machine (thus milling the material) equipped with pump, which pours dispersion liquid at high voltage and high speed continuously in the homogenous valve (see Abstract). Imazawa et al also teach in accordance with a conventional method, separation removal of extraction slag and/or the juice slag is carried out using a liquid cyclone, a clarifier, centrifugal separation (thus insoluble fibers are removed by centrifuging the carrier after milling), filatration, precision filtration, decantation etc [0027] (see machine translation attached). Imazawa et al teach the method is suitable for the continuous mass production and extremely effective from the viewpoint of the effective utilization of food resources and the economic merit compared with conventional extraction/squeezing method (see Abstract).

First of all, the MPEP states the following: "[E]ven though product-by-process claims are limited by and defined by the process determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process...The product-by-process claim was rejected because the end product, in both the prior art and the allowed process, ends up containing metal carboxylate. The fact that the metal carboxylate is not

directly added, but is instead produced in-situ does not change the end product" (see MPEP 2113 [R-1]). Therefore, although Osanai teaches using strainers twice, instead of using claimed centrifuging process, insoluble fibers are being removed either way, and the final products are not materially different. Even if there is subtle difference between using strainers and centrifuge machine, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use the claimed centrifuging step since Imazawa et al teach removing extraction slag by a liquid cyclone, a clarifier, centrifugal separation, filatration, precision filtration, or decantation. It is evidenced by Imazawa et al that centrifuging step is well known in the art to remove extraction slags, and it is used interchangeably in the art with other methods such as filtration or straining. Since Imazawa et al teach using dispersion medium cowsmilk to grind raw plant material for extraction, and since Imazawa et al teach the method is extremely effective in utilization of food resources and has economic merit compared with conventional extraction/squeezing method, one of the ordinary skills in the art would have been motivated to combine the teachings of the references together.

From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Claims 1-8, 12-14, and 20-28 are newly rejected under 35 U.S.C. 103(a) as being unpatentable over Osanai, Edenharder et al, Faulks et al, Hovari et al, and Imazawa et al as

applied to claims 1-8, 14, 20, and 21 above, and further in view of Hong et al (KR 2003022942 A).

This is a new rejection necessitated by the Applicant's amendment filed on 5/11/2011.

The teachings of Osanai, Edenharder et al, Faulks et al, Hovari et al, and Imazawa et al are set forth above and applied as before.

The combination of Osanai, Edenharder et al, Faulks et al, Hovari et al, and Imazawa et al do not specifically teach a freeze-dried powder; neither the combination explicitly teach using a plant-based milk carrier such as soymilk.

Hong et al teach provided is a process for preparing liquid and powder types of fermented vegetable milk using legumes and rice as main ingredients to improve its preservability and distribution. Hong et al teach the process for preparing liquid type of fermented vegetable milk is characterized by culturing a mixture of soy milk (thus a plant-based milk carrier) and rice milk with bifidobacterium and Lactobacillus sp. strains and fermenting it, wherein the mixing ratio of soy milk to rice milk is 1:10-10:1, the rice milk is obtained by saccharifying polished or unpolished rice or a mixture thereof. The powder type of fermented vegetable milk is manufacture by freeze-drying the prepared liquid type of vegetable milk to minimize the destroy of nutrients (see Abstract).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use freeze-dried powder in the composition in Osanai since Hong et al teach vegetable milk is manufactured by freeze-drying the prepared liquid type of vegetable milk to minimize the destroy of nutrients. Therefore, one of ordinary skill in the art would have been

motivated to use freeze-dried powder in the composition in Osanai to minimize the destroy of nutrients.

It would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use a plant-based milk carrier such as soymilk since Hong et al teach a vegetable milk using legumes and rice as main ingredients to improve its preservability and distribution. In addition, as evidenced by Osanai a "regulated soymilk" (thus a plant-based milk carrier) is well known in the art by the time the invention was made. Therefore, one of ordinary skill in the art would have been motivated to use plant-based milk carrier to improve its preservability and distribution.

From the teachings of the references, it is apparent that one of the ordinary skills in the art would have had a reasonable expectation of success in producing the claimed invention.

Thus, the invention as a whole is *prima facie* obvious over the references, especially in the absence of evidence to the contrary.

Applicant argues that "Applicants have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and oil-soluble bioactives of the plant material. Moreover, Applicants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and

further provides the claimed Composition as a whole to be stable, miscible and dispersible in an aqueous system. See specification, page 2, lines 22-28 and page 3, lines 6-11" (page 8, last paragraph).

This is not found persuasive. According to MPEP 716.02 (a), a greater than additive effect is not necessarily sufficient to overcome a prima facie case of obviousness because such an effect can either be expected or unexpected. Applicants must further show that the results were greater than those which would have been expected from the prior art to an unobvious extent, and that the results are of a significant, practical advantage. *Ex parte* The NutraSweet Co., 19 USPQ2d 1586 (Bd. Pat. App. & Inter. 1991). In the instant case, Applicant needs to present a side by side comparison between the claimed invention and the closest art to show the allegedly surprising results, mere argument or allegation is insufficient to overcome the obviousness rejection.

Applicant argues that "*Osanai, Edenharder, Faulks, Hovari* and *Imazawa* fail to disclose or suggest each and every element of independent Claims 1, 12 and 14. *Osanai, Edenharder, Faulks, Hovari* and *Imazawa* alone or in combination fail to disclose or suggest a miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as required by independent Claims 1, 12 and 14" (page 9, 2nd paragraph).

This is not found persuasive. Osanai teaches a method of producing cowsmilk containing vegetables characterized as placing approximately 15 g of carrots, approximately 22.2 g of lemon, and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing it and mixing it, straining it in a strainer twice (thus excluding insoluble fibers), and then adding cowsmilk to this so that it reaches 200 cc. It is noted that since the vegetables were

mixed with milk, pulverized, and strained, thus the vegetables are miscible and dispersible in the aqueous milk system. Regarding the limitation "stable", please see the 112, 2nd rejection stated above.

Applicant argues that "*Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. See *Osanai,* pages 5-6. To distinguish the composition of *Osanai* with that of the claimed compositions, Applicants submit herewith a Declaration under 37 C.F.R. §1.132 *("Declaration")* that demonstrates the deficiencies of the prior art with respect to the present claims (page 9, 2nd paragraph). Applicant argues that "As supported by the *Declaration, Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. Each of the embodiments of the beverage disclosed by *Osanai* at least includes approximately 22.5 grams of lemon. Moreover, lemon is an essential aspect of *Osanai's* beverage as it supplies vitamin C in an amount that is not satisfied with the remaining elements of the beverage. See *Osanai,* paragraph 12" (page 9, 3rd paragraph). Applicant argues that "As supported by the *Declaration,* an experiment was performed to determine the impact of lemon on cow's milk as taught by *Osanai.* The experiment showed that the addition of 22.5 grams of lemon to 100 ml of milk led to a precipitation/coagulation of a large portion of the milk proteins in the milk causing an obvious lack of miscibility. See Exhibit A of the *Declaration.* Therefore, upon experimental testing to compare *Osanai's* beverage against the claimed invention, it is clear that *Osanai* does not provide a miscible primary composition that is stable, miscible and dispersible in an aqueous system according to the claimed invention" (page 9, 4th paragraph). Applicant argues that "As supported by the *Declaration,* the inventors have surprisingly found that the milk proteins are essential for the

improved extraction of the lipophilic bioactive components according to the claimed invention. The claimed miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system provides the optimal conditions for extracting the most lipophilic bioactive components from plant materials. In contrast, because of the precipitation/coagulation of a large portion of the milk proteins in the beverage of *Osanai,* these precipitated or coagulated proteins are immiscible in solution and are no longer free to extract the lipophilic bioactive components of plant materials. This reduces the effectiveness of the extraction and the amount of the extracted bioactive components that could end up in the beverage. As a result, the miscible primary composition of the claimed invention is a distinguishable product over the immiscible beverage resulting from the components and process of *Osanai*" (page 9, last paragraph bridging page 10).

The Declaration under 37 CFR 1.132 filed on 5/11/2011 is insufficient to overcome the 103 rejection as set forth in the last Office action because: In Exhibit A, 22.5 g lemon was mixed with 100 ml cow's milk, and extract cow's milk was adjusted to 200 ml, protein precipitate was observed in 10 minutes. However, this has nothing to do with the cited reference Osanai. First of all, Osanai does not teach a composition comprising only lemon and cow's milk as shown in Exhibit A. Secondly, the Exhibit A in the Declaration does not have the process of "pulverizing it and mixing it, straining it in a strainer twice" as taught by Osanai. Furthermore, the Declaration does not have a negative control, for instance, the claimed composition does not have any precipitation as a comparison.

Applicant argues that "Applicants also respectfully submit that the skilled artisan would have no reason to combine the cited references to arrive at the present claims because the cited

references are directed to unrelated products that have completely different objectives. *Osanai* is entirely directed to cow's milk containing vegetables whose main constituent is rapa gourd, wherein the vegetable containing rapa gourd is mixed with cowsmilk. See *Osanai,* pages 5-6. *Edenharder* is entirely directed to the isolation and characterization of antimutagenic flavonoids from spinach. See *Edenharder,* Abstract. Indeed, the entire disclosure of *Edenharder* is directed to the purification of antimutagens from spinach by preparative and micropreparative HPLC from a methanol/water extract of dry spinach after removal of lipophilic compounds. *Id.* As such, not

only is the subject matter of *Edenharder* nonanalogous art when compared to *Osanai* and the present claims, but *Edenharder* teaches away from the present claims when *Edenharder* discloses removal of lipophilic compounds from the spinach extract" (page 10, 2nd paragraph). Applicant argues that "Similar to *Edenharder, Faulks* is entirely directed to the quantification of 13-carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modeling of triglyceride-rich lipoprotein plasma fraction. See *Faulks,* Summary. Like *Edenharder,* the green vegetable *of Faulks* is spinach and the entire disclosure is directed to the kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals. See *Faulks,* Summary and Introduction. As such, *Faulks* is also nonanalogous art when compared to *Osanai* and the present claims" (page 10, 3rd paragraph). Applicant argues that "*Hovari* is entirely directed to the effects of flavanoids on human health and the content of flavonoids in specific vegetables. See *Hovari,* Introduction, Table 1. *Imazawa* is entirely directed to extraction efficiency and preparation of juice in a short time for industrialization. See *Imazawa,* paragraphs

18 and 19. *Imazawa* discloses processes that include pulverizing coffee beans, fruits, vegetables, etc., adding a dispersing media to the pulverized coffee beans, fruits, vegetables, etc., and then homogenizing the mixture. See *Imazawa,* Working Examples" (page 10, last paragraph bridging page 11). Applicant argues that "As such, the cited references are clearly directed to unrelated products or processes that have completely different objectives. Moreover, none of the cited references even recognizes the benefits obtained by the presently claimed compositions including, for example, improved bioavailability and miscibility of from extracted fruits or plant materials by milling the material in a milk or milk protein-containing carrier and centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials to remove the insoluble fibers. Such treatments allow the essential lipophilic and hydrophilic bioactive components to have improved bioavailability and miscibility in the milk or milk protein-containing carrier. See specification, page 4, lines 1-3" (page 11, 2nd paragraph).

This is not found persuasive. The rejection is based on Osanai in view of Imazaawa, references Edenharder et al, Faulks et al, and Hovari et al are only brought in to show the intrinsic properties of the product in Osanai. Osanai teaches "the cow's milk containing the vegetable is prepared by placing about 12.5 g KOMATSU-NA, about 2.5 g spinach and about 2.5 g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10 cc cow's milk in a mixer, pulverizing (thus milling in milk) and mixing the ingredients" (see Abstract). The process of mixing the claimed ingredient with milk in a mixer, pulverizing, and mixing the ingredient is not materially different from the claimed "milling the material in the milk or milk protein-containing carrier". Although Osanai teaches using strainers twice, instead of using claimed centrifuging process, insoluble fibers are being removed either way, and the final

products are not materially different. Even if there is subtle difference between using strainers and centrifuge machine, it would have been *prima facie* obvious for one of ordinary skill in the art at the time the invention was made to use the claimed centrifuging step since Imazawa et al teach removing extraction slag by a liquid cyclone, a clarifier, centrifugal separation, filatration, precision filtration, or decantation. It is evidenced by Imazawa et al that centrifuging step is well known in the art to remove extraction slags, and it is used interchangeably in the art with other methods such as filtration or straining. Since Imazawa et al teach using dispersion medium cowsmilk to grind raw plant material for extraction, and since Imazawa et al teach the method is extremely effective in utilization of food resources and has economic merit compared with conventional extraction/squeezing method, one of the ordinary skills in the art would have been motivated to combine the teachings of the references together.

Applicant argues that "Finally, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there exists no reason for the skilled artisan to make the proposed modification. *In re Gordon,* 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). In fact, Applicants submit that what the Patent Office has done here is to apply hindsight reasoning by attempting to selectively piece together teachings of each of the references in an attempt to recreate what the claimed invention discloses. Indeed, the skilled artisan must have a reason to combine the cited references to arrive at the present claims. Applicants respectfully submit that such a reason is not present in the instant case" (page 11, 3rd paragraph).

In response to Appellant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on

obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the Appellant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Qiuwen Mi whose telephone number is 571-272-5984. The examiner can normally be reached on 8 to 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on 571-272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Qiuwen Mi/

Primary Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 10/598,909	Applicant(s)/Patent Under Reexamination WANG ET AL.	
	Examiner QIUWEN MI	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-			
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	KR 2003022942 A	03-2003	Korea, Republic	HONG H O et al.	----------
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

EXHIBIT D

PUB-NO: JP409107880A
DOCUMENT-IDENTIFIER: JP 09107880 A
TITLE: COW'S MILK CONTAINING VEGETABLE AND ITS PRODUCTION

PUBN-DATE: April 28, 1997

INVENTOR-INFORMATION:

NAME	COUNTRY
OSANAI, KAORU	

ASSIGNEE-INFORMATION:

NAME	COUNTRY
OSANAI KAORU	
OSANAI KENJI	
OSANAI MIO	

APPL-NO: JP07298842
APPL-DATE: October 24, 1995

INT-CL (IPC): A23C 9/152

ABSTRACT:

PROBLEM TO BE SOLVED: To produce a suitably producible cow's milk at a low cost by using a widely used vegetable, capable of enriching iron, enhancing hematopoietic actions, further containing various vitamins or minerals blended in good balance and effective against various symptoms of anemia, constipation or climacteric disturbance of women.

SOLUTION: This cow's milk contains a vegetable and is obtained by adding about 12.5g KOMATSU-NA [Brassica campestris (rapa groupl), about 2.5g spinach, about 2.5g total amount of mulukkiyya, parsley, water cress and beefsteak plant, 22.5g lemon and 2.5g reducing palatinose with about 150cc cow's milk. Furthermore, the cow's milk containing the vegetable is prepared by placing about 12.5g KOMATSU-NA, about 2.5g spinach and about 2.5g total amount of mulukkiyya, parsley, water cress and beefsteak plant based on 10cc cow's milk in a mixer, pulverizing and mixing the ingredients, adding about 22.5g lemon and about 2.5g reducing palatinose thereto and further adding cow's milk thereto so as to make the sum total to 200cc.

PTO 10-0251

CC=JP
DATE=19970428
KIND=A
PN=09107880

COWSMILK CONTAINING VEGETABLES AND ITS PRODUCTION
[YASAI-IRI GYUNYU TO SONO SEIZO HOHO]

KAORU OSANAI

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. OCTOBER 2009
TRANSLATED BY: SCHREIBER TRANSLATIONS, INC.

PUBLICATION COUNTRY	(10): JP
DOCUMENT NUMBER	(11): 09107880
DOCUMENT KIND	(12): A
PUBLICATION DATE	(43): 19970428
APPLICATION NUMBER	(21): 07298842
APPLICATION DATE	(22): 19951024
INTERNATIONAL CLASSIFICATION	(51): A 23 C 9/152
PRIORITY COUNTRY	(33): NA
PRIORITY NUMBER	(31): NA
PRIORITY DATE	(32): NA
INVENTOR(S)	(72): OSANAI, KAORU
APPLICANT(S)	(71): KAORU, OSANAI; KENJI OSANAI; MIYU OSANAI
DESIGNATED CONTRACTING STATES	(81):
TITLE	(54): COWSMILK CONTAINING VEGETABLES AND ITS PRODUCTION
FOREIGN TITLE	[54A]: YASAI-IRI GYUNYU TO SONO SEIZO HOHO

[Scope of Patent Claims]

[Claim 1]

Cowsmilk containing vegetables characterized as mixing approximately 12.5 g of rapa gourd [*Brassica campestris*]; approximately 2.5 g of spinach; a total of approximately 2.5 g of *mulukkiyya*, parsley, watercress, and *shiso* [beefsteak plant]; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose in 150 cc of cowsmilk.

[Claim 2]

A method of producing cowsmilk containing vegetables characterized as placing approximately 12.5 g of rapa gourd [*Brassica campestris*]; approximately 2.5 g of spinach; a total of approximately 2.5 g of *mulukkiyya*, parsley, watercress, and *shiso* [beefsteak plant]; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose in 100 cc of cowsmilk in a mixer,

pulverizing it, and mixing it, and adding more cowsmilk to this so that there is a total of 200 cc.

[Claim 3]

Cowsmilk containing vegetables characterized as mixing approximately 20 g of rapa gourd [*Brassica campestris*]; a small amount of parsley; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose in approximately 150 cc of cowsmilk.

[Claim 4]

A method of producing cowsmilk containing vegetables characterized as placing approximately 20 g of rapa gourd [*Brassica campestris*]; a small amount of parsley; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing and mixing it, and adding cowsmilk to this so that it reaches 200 cc.

[Claim 5]

Cowsmilk containing vegetables characterized as mixing approximately 15 g of carrots; approximately

22.5 g of lemon; and approximately 2 g of reduced palatinose to approximately 195 cc of cowsmilk.

[Claim 6]

A method of producing cowsmilk containing vegetables characterized as placing approximately 15 g of carrots; approximately 22.2 g of lemon; and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing it and mixing it, straining it in a strainer twice, and then adding cowsmilk to this so that it reaches 200 cc.

[Detailed Description of the Invention]

[0001]

[Technical Field]

The present invention relates to cowsmilk containing vegetables and particularly to cowsmilk wherein vegetables whose main constituent is rapa gourd (*Brassica campestris*) are mixed with cowsmilk or cowsmilk wherein vegetables whose main constituent is carrot is mixed with cowsmilk which is effective in

treating anemia and the like as well as to a method for producing it.

[0002]

[Prior Art]

Cowsmilk contains a great many nutrients including a variety of vitamins. It has many calories and is widely consumed as it has an outstanding nutritional balance. Further, it contains many nutrients so that calcium is mixed with it. As a result, the daily requirement of calcium can be taken from the milk or there is a variety of so-called fortified cowsmilk to which vitamins and iron have been added. It is also known as a so-called mixed juice drink in which *mulukkiyya* and spinach, celery, parsley and a variety of other greenish yellow vegetables, apples, muscat grapes, grapefruit and other juices are mixed.

[0003]

[Problems Which the Present Invention is Intended to Solve]

However, although these have their own characteristics, even if they contain fortified iron and promote a hematopoietic action, merely reinforcing the amount of iron is not still not effective in treating anemia which frequently occurs in women and there is a need for cowsmilk containing constituents which are effective for these symptoms.

[0004]

As a result, it is an object of the present invention to provide cowsmilk containing vegetables which fortifies the iron content thereby enhancing the hematopoietic action, which compounds a variety of vitamins and minerals so that they are properly balanced, which is effective in anemia in women and in constipation and a variety of symptoms in women's climacteric, which uses vegetables which are widely

consumed so that it can be manufactured inexpensively and reasonably.

[0005]

[Means Used to Solve the Problems]

In order to solve the abovementioned problems, the present invention consists of cowsmilk containing vegetables by mixing approximately 12.5 g of rapa gourd (*Brassica campestris*); approximately 2.5 g of spinach; a total of approximately 2.5 g of *mulukkiyya*, parsley, watercress and *shiso*; 22.5 g of lemon; and 2.5 g of reduced palatinose to approximately 150 cc of cowsmilk. The method of producing the cowsmilk containing these vegetables involves placing approximately 12.5 g of (*Brassica campestris*); approximately 2.5 g of spinach; and a total of approximately 2.5 g of *mulukkiyya*, parsley, watercress and *shiso* in 100 cc of cowsmilk in a mixer, pulverizing and mixing them, adding to this approximately 22.5 of lemon and approximately 2.5 g of

reduced palatinose, and adding cowsmilk to this so it reaches a total of 200 cc. It also consists of cowsmilk containing vegetables made by mixing approximately 20 g of rapa gourd (*Brassica campestris*); a small amount of parsley; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose with approximately 150 cc of cowsmilk. It also consists of a method for producing cowsmilk containing these vegetables involving adding approximately 20 g of rapa gourd (*Brassica campestris*); a small amount of parsley; approximately 22.5 g of lemon; and approximately 2.5 g of reduced palatinose to 100 cc of cowsmilk in a mixer, pulverizing and mixing it, and adding milk to this so that it reaches 200 cc. It also consists of cowsmilk containing vegetables made by mixing approximately 15 g of carrots; approximately 22.5 g of lemon; and approximately 2 g of reduced palatinose; as well as a method of producing cowsmilk containing these vegetables which involves placing approximately 15 g

of carrots; approximately 22.5 g of lemon; and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer, pulverizing and mixed these, straining it twice using a strainer, adding cowsmilk to this so that it reaches a capacity of 200 cc.

[0006]

The present invention is configured as indicated above so that it promotes the proliferative action for the blood by including rapa gourd (*Brassica campestris*) which has large amounts of iron as a natural food, which is effective in treating anemia, which also contains *mulukkiyya* and watercress so that vitamins and calcium and a variety of other minerals can be contained, which acts synergistically with the constituents in the vitamins in the cowsmilk thereby providing cowsmilk which contains vegetables. Moreover, it is rich in calcium, iron, vitamin A and vitamin C and has a variety of other effects and actions, which mixes in rapa gourd which is easy to

drink so that it acts synergistically with the cowsmilk, thereby providing a drink having a high nutritional value. Moreover, it has large amounts of beta carotene which changes into vitamin A in the body and acts synergistically with the constituents in the cowsmilk, which utilizes the activation of the

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function of the cancer prevention and the stomach, thereby providing a drink which is high in nutrients.

[0007]

[Mode of Working the Present Invention]

(Practical Example 1)

We mixed rapa gourd (*Brassica campestris*) and a variety of other vegetables with the cowsmilk. When preparing a total of 200 cc of cowsmilk containing vegetables, the following constituent materials in the practical examples should be used: 150 cc of cowsmilk; 12.5 g of rapa gourd (*Brassica campestris*); 2.5 g of spinach; approximately 2

mulukkiyya leaves; small amounts of watercress, parsley and *shiso* totaling 2.5 g; 22.5 g of lemon; and 2.5 g of reduced palatinose.

[0008]

Of the abovementioned constituents, cowsmilk contains a variety of nutrients and large amounts of calories and maintains a good nutritional balance so that it used as the main constituent. Rapa gourd (*Brassica campestris*) is an annual grass of the oil and plant family and is a variety of *yuna*. It contains particularly large amounts of iron as well as large amounts of calcium. It contains other nutrients and is easy to drink. It has been reported that the rapa gourd (*Brassica campestris*) is effective in suppressing the canceration of cells. It is also effective in treating pyorrhea and is said to be effective in treating atopic dermatitis and hypertension. In traditional Asia medicine, it is said to warm the stomach, increase its function thereby

facilitating evacuation and urination. It is also said to be useful in improving sensitivity to cold and dizziness.

[0009]

Spinach is an annual and biennial plant belonging to *Chenopodioideae* family and is known to be a vegetable supplying inorganic nutrients and vitamins. It has twice the amount of vitamin C in lemons and is also rich in beta carotene and vitamin B_1 and functions to facilitate the body metabolism. It is also a vegetable which contains large amounts of iron and is effective in preventing anemia. It also contains trace amounts of zinc and the like, eliminates impairments of the taste buds and is said to promote insulin accumulation. Spinach is effective in maintaining the health of the stomach and thorax. It is also effective in stress-related hypertension and constipation in elderly persons and is said to be effective as a styptic. It also quenches thirst

brought on by diabetes due to the action of the zinc, regulates urination and promotes normalization of blood sugar.

[0010]

Mulukkiyya is an annual plant belonging to the *Corchorus* genus of the *Tiliaceae* family. It is a greenish yellow plant cultivated in the Arab tropical zone mainly in Egypt and is a type of edible plant. This plant is rich in a variety of vitamins and calcium and a variety of other minerals. Recently a great deal of it has been cultivated in Japan. When 200 cc of cowsmilk containing vegetables in the present invention is prepared, approximately two standard *mulukkiyya* leaves are used. Moreover, watercress is a perennial aquatic plant of the *Cruciferae* family having a slight saltiness and odor and is rich in calcium, iron and other minerals and vitamin A and C. Parsley is a biennial or perennial plant of the dropwort family whose leaves are used as

a food. It has a strong odor and bitterness and is rich in nutrients. For example, as much as 7500 micrograms of carotene are contained in 100 g of it. It has a bactericidal action and is used in traditional Chinese herbal medicine as an antitoxic remedy and for treatment of eczema, pimples and the like. Its distinctive odor and bitterness act on the stomach, increase peristalsis of the stomach, activate secretion of bile so that digestion is promoted and is said to function to promote the appetite and to help in recovering from fatigue.

[0011]

Shiso (beefsteak plant) is an annual plant belonging to the *Perilla* family whose sprouts, spikes and leaves contain cyanidine glycoside having an anti-allergic action. *Shiso* has been used since ancient times to treat urticaria caused by eating shellfish and meat. Its effect even on pollinosis and allergic rhinitis, atopic dermatitis and the like has been

clarified by the presence of the abovementioned glycoside. It is used in Chinese herbal medicine to treat colds and it has been found to have an action in warming the body for shisoaldehyde in refined oil constituents as well as limonene, pinene and the like. As a result, it is used to improve the functioning of the stomach and to treat stomachache and diarrhea. *Shiso* is also said to be effective in anemia and skin eruptions. These vegetables are rich in a variety of vitamins and calcium and other minerals as indicated above, they have a distinctive flavor so that they are used in small quantities.

[0012]

Lemon is an evergreen fruit-bearing plant containing large amounts of vitamin C. It is used to provide vitamin C not satisfied in the abovementioned constituents. Reduced palatinose is a sweetener and makes the cowsmilk containing vegetables in the present invention easy to drink without using sugar.

Since no sugar is mixed in to this, it is suitable for treating obesity and as a means of preventing tooth decay and can be consumed easily without having to worry about these matters. Table 1 indicates the main constituents per 100 g of the abovementioned vegetables.

[0013]

[Table 1]

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Table 1

Constituent	**A**	**B**	**C**	**D**	**E**	**F**	**G**
energy kcal							
protein g							
calcium mg							
iron mg							
potassium mg							
zinc μm							
vitamin A efficacy IU							
vitamin B_1 mg							
vitamin B_2 mg							
vitamin C mg							
vitamin E efficacy mg							
edible fiber g							

A: rapa gourd (*Brassica campestris*); B: spinach; C: *mulukkiyya*; D: watercress; E: parsley; F: *shiso* (beefsteak plant); G: carrot

(See original for values)

[0014]

The cowsmilk containing vegetables in the present invention made up of the abovementioned materials contains the constituents indicated in Table 2 and has been corroborated in the analytical test result tables of the Japan Food Center. Furthermore, Table 1 indicates the comparative examples. “Z standard cowsmilk” is standard cowsmilk from company Z. “Y fortified cowsmilk” is commonly known as “fortified cowsmilk” from company Y with fortified calcium in standard cowsmilk. “A regulated soymilk” is commonly known as “regulated soymilk” from company A wherein the soymilk has been regulated. “B fruit juice drink” is a drink containing fruit made into a juice, from company B. “C vegetable drink” is a vegetable drink

from company C. For reference, the constituents per 190 g can are: 22 *mulukkiyya* leaves; 3 pieces of spinach; 11 g of carrot; 4 g of celery; 2 g of parsley; 2 g of cabbage; 2 g of green pepper; 2 g of green peas; a small amount of watercress; a small amount of radish; a small amount of clover leaves; 2 or 3 apples; 11 g of muscat grapes; 11 g of grapefruit; and a small amount of lemon.

[0015]

[Table 2]

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Constituent	**A**	**B**	**C**	**D**	**E**	**F**
energy kcal						
protein g						
lipid g						
carbohydrate g						
ash content						
calcium mg						
iron mg						
total carotene mg						
retinol μm						
vitamins mg						
A IU						
C mg						

A: The present invention; B: Z standard cowsmilk; C: Y fortified cowsmilk; D: A adjusted soymilk; E: B fruit juice drink; F: C vegetable milk

See original for values]

[0016]

When preparing the cowsmilk containing the abovementioned vegetables, we placed 12.5 g of rapa gourd (*Brassica campestris*); 2.5 g of spinach; and a total of 2.5 g of *mulukkiyya*, parsley, watercress and *shiso* in 100 cc of cowsmilk in a mixer, operated the mixer and pulverized and mixed these. To this we added 22.5 g of lemon and 2.5 g of reduced palatinose, at the same time, adding cowsmilk to make up the residual capacity so that the overall capacity was 200 cc. We stirred it again and mixed it, thereby providing the cowsmilk containing vegetables.

[0017]

(Practical Example 2)

We mixed mainly rapa gourd (*Brassica campestris*) with the cowsmilk and produced a nutritious milk drink. When preparing a total of 200 cc of cowsmilk containing vegetables, the following constituent materials in this practical example should be used: 150 cc of cowsmilk; 20 g of rapa gourd (*Brassica campestris*); a small amount of parsley; 22.5 g of lemon; and 2.5 g of reduced palatinose.

[0018]

Of the constituents indicated above, the rapa gourd (*Brassica campestris*) is rich in calcium, iron, vitamin A and vitamin C, has a variety of other effects and actions and is easy to drink. When cowsmilk is mixed with this, it acts synergistically with the cowsmilk constituents thereby providing a highly nutritious drink.

[0019]

When preparing the abovementioned cowsmilk containing vegetables, we placed 20 g of rapa gourd (*Brassica campestris*);, a small amount of parsley; 22.5 g of lemon; and 2.5 g of reduced palatinose to 100 cc of cowsmilk in a mixer, operated the mixer and pulverized and mixed these. Next, we added cowsmilk to fill the remaining capacity so that the total capacity was 200 cc and we again stirred it and mixed it.

[0020]

(Practical Example 3)

We mainly mixed carrot with the cowsmilk and obtained a healthy milk drink. 195 cc of cowsmilk; 15 g of carrots; 22.5 g of lemon; and 2 g of reduced palatinose should be used as the constituent materials in this practical example.

[0021]

Of the constituents indicated above, carrots are an annual and biennial grass belonging to the Japanese

parsley family. They contain a large amount of beta carotene which changes to vitamin A in the body. They are used to prevent cancer, to activate the function of the stomach and are also good for eye health. When cowsmilk is mixed with this, it acts synergistically with the constituents in the cowsmilk thereby providing a highly nutritious drink. It is rich in a variety of vitamins with the exception of vitamin C and contains large amounts of potassium, calcium, sulfur, phosphorus and other minerals.

[0022]

One method of preparing cowsmilk containing vegetables involves placing 15 g of carrots, approximately 22.5 g of lemon and approximately 2 g of reduced palatinose in 100 cc of cowsmilk in a mixer and pulverizing and mixing them. We strained the reduced palatinose in a strainer twice and added cowsmilk to this so that it reached a total of 200 cc.

[0023]

Furthermore, we tested the cowsmilk containing vegetables in the present invention containing the abovementioned constituents on the inventor who had been anemic for the past 20 years who had test drunk this continuously for a long period of time. As a result, the symptoms of anemia reappeared when the inventor stopped test-drinking this. He test-drank it again and there was no anemia and he remains free of anemia to the present. Moreover, after test drinking the cowsmilk containing the vegetables, not only were there no symptoms or side effects but he was regular. Now that he is 60 years old, there are no particular symptoms of climacteric and he remains symptom-free to this day. The cowsmilk containing vegetables has been proved to be effective as a drink used to prevent climacteric-related impairments.

[0024]

[Effect of Invention]

The cowsmilk containing vegetables in the present invention is made up of the abovementioned materials

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and is produced as indicated above. As a result, the iron is fortified and the hematopoietic action is enhanced. Further, the various vitamins and minerals are compounded so that there is a good balance. It is effective in anemia in women as well as constipation, climacteric impairments and other symptoms. By using vegetables which are widely available, it can be produced at low cost and easily at home merely by using an ordinary mixer. Manufacturing in factories requires very simple equipment and it can be easily manufactured.

EXHIBIT E

Isolation and Characterization of Structurally Novel Antimutagenic Flavonoids from Spinach (*Spinacia oleracea*)

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Thirteen compounds, isolated from spinach (*Spinacia oleracea*), acted as antimutagens against the dietary carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline in *Salmonella typhimurium* TA 98. The antimutagens were purified by preparative and micropreparative HPLC from a methanol/water (70:30, v/v) extract of dry spinach (commercial product) after removal of lipophilic compounds such as chlorophylls and carotenoids by solid-phase extraction (SPE). Pure active compounds were identified by instrumental analysis including FT-IR, ^{1}H and ^{13}C NMR, UV−vis spectroscopy, and mass spectrometry. All of these compounds were flavonoids and related compounds that could be attributed to five groups: (A, methylenedioxyflavonol glucuronides) 5,3′-dihydroxy-4′-methoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (compound **1**), 5,2′,3′-trihydroxy-4′-methoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (compound **2**), 5-hydroxy-3′,4′-dimethoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (compound **3**); (B, flavonol glucuronides) 5,6,3′-trihydroxy-7,4′-dimethoxyflavonol 3-*O*-β-glucuronide (compound **4**), 5,6-dihydroxy-7,3′,4′-trimethoxyflavonol 3-*O*-β-glucuronide (compound **5**); (C, flavonol disaccharides) 5,6,4′-trihydroxy-7,3′-dimethoxyflavonol 3-*O*-disaccharide (compound **6**), 5,6,3′,4′-tetrahydroxy-7-methoxyflavonol 3-*O*-disaccharide (compounds **7** and **8**); (D, flavanones) 5,8,4′-trihydroxyflavanone (compound **9**), 7,8,4′-trihydroxyflavanone (compound **10**); (E, flavonoid-related compounds) compounds **11**, **12**, and **13** with incompletely elucidated structures. The yield of compound **1** was 0.3%, related to dry weight, whereas the yields of compounds **2**−**13** ranged between 0.017 and 0.069%. IC_{50} values (antimutagenic potencies) of the flavonol glucuronides ranged between 24.2 and 58.2 μM, whereas the flavonol disaccharides (compounds **7** and **8**), the flavanones (compounds **9** and **10**), and the flavonoid-related glycosidic compounds **11**−**13** were only weakly active. The aglycons of compounds **7** and **8**, however, were potent antimutagens (IC_{50} = 10.4 and 13.0 μM, respectively).

Keywords: *Spinach; 2-amino-3-methylimidazo[4,5-f]quinoline; flavonoids; antimutagenic activity; Salmonella/reversion assay*

INTRODUCTION

In more than 200 case-control and several human cohort studies it was shown that a high consumption of fruits and vegetables is consistently associated with a low incidence for all common cancer sites except for hormone-dependent breast and prostate cancers (*1*−*4*). On the whole, epidemiological evidence is now considered overwhelming (*5*), and particularly strong associations were detected for cancers of the alimentary and respiratory tracts. Associations exist for a wide variety of fruits and vegetables, although available evidence suggests some differences for specific sites (*1*). In model experiments with rodents, protective effects of common vegetables of the human diet, mostly cruciferous ones, against the induction of cancer have been found, confirming the epidemiological evidence (*6*). Similar results have also been obtained in less expensive short-term assays: In the in vivo mouse bone-marrow micronucleus assay various fruits and vegetables reduced clastogenic activities of model compounds (*7*, *8*), and in the in vitro *Salmonella*/reversion assay a great number of fruits and vegetables exerted protective effects against mutagenicity induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline, other heterocyclic amines from cooked food, and polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene (*9*−*12*). These compounds are carcinogenic in laboratory animals and possibly in humans, too. In our investigations particularly strong and consistent anticlastogenic and antimutagenic effects of spinach against 2-amino-3-methylimidazo[4,5-*f*]quinoline were seen, although the relevant active compounds were largely unknown. The objective of this study was to identify compounds with antimutagenic potential against this imidazoquinoline in the *Salmonella*/reversion assay among the multitude of compounds present in spinach by activity-directed enrichment, followed by purification and spectroscopic identification.

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MATERIALS AND METHODS

Materials. Dry powders of spinach (*Spinacia oleracea*) were obtained from Bestfoods Europe, Heilbronn, Germany. The mutagen 2-amino-3-methylimidazo[4,5-*f*]quinoline was pur-

10.1021/jf0013712 CCC: $20.00
Published on Web 05/11/2001

chased from Toronto Research Chemicals, Downsview, ON, Canada. Reference compounds such as chlorophyll, carotenoids, and flavonoids were obtained from Roth, Karlsruhe, Germany, or Fluka, Neu-Ulm, Germany; sugar standards as well as galacturonic and glucuronic acids were from Aldrich, Steinheim, Germany. Glucose-6-phosphate and NADP were purchased from Boehringer (Roche), Mannheim, Germany. All solvents including dimethyl sulfoxide (DMSO) as well as chemicals not specifically indicated were from Merck, Darmstadt, Germany. Solvents were of the highest grade necessary, pro analysi, chromatographically pure or gradient quality.

Extraction and Isolation. The dry spinach powder (100 g) was extracted twice with 10 volumes of methanol/water (70:30, v/v) at 40 °C for 2 h by stirring, and the extracts were filtered and combined. By this procedure, a maximum of materials (~5.5 g/100 g) could be extracted with maximum amounts of flavonoids present (45% flavonoids, 10% chlorophylls, 10% epiphasic carotenoids, 35% hypophasic carotenoids). With other solvents and solvent mixtures such as ethanol/water (50:50, v/v), 2-propanol/water (50:50, v/v), methanol, ethanol, 2-propanol, and *n*-hexane the contributions of flavonoids were 35, 35, 35, 10, 10, and 0%, respectively, whereas the amounts of chlorophylls and carotenoids increased appropriately. Soxhlet extraction was not superior to the batch procedure, neither on a mass basis nor when related to antimutagenic activities. Application of the solvents hexane, dichloromethane, acetone, and 2-propanol in the sequence of polarity as performed in previous investigations with plant residues (*12*) resulted in 40−70% overlapping of selectivity. To remove chlorophylls and carotenoids, up to 30 g of a reversed phase packing, ODS-C_{18} (Baker, Gross-Gerau), was added to the aqueous/methanolic spinach extract, shaken for 30 min, and then centrifuged. The solid material was washed twice with 2 volumes of methanol/water (70:30) and the washings were combined with the original extract. This solution was concentrated under reduced pressure to remove methanol and a part of the water, then frozen at −20 °C, and freeze-dried to protect potentially sensitive compounds.

Evaluation of Antimutagenicity against 2-Amino-3-methylimidazo[4,5-*f*]quinoline and Related Procedures. The antimutagenicity of extracts, HPLC fractions, and compounds was evaluated as reported previously (*11*−*13*). The following components were added in order: 500 μL of isotonic KCl, 20 ng of mutagen (2353 ± 411 revertants/plate), dissolved in 50 μL of dimethyl sulfoxide, 200 μL of solution of test compound in dimethyl sulfoxide, 500 μL of S9 mix, 100 μL of bacterial suspension [the original broth was centrifuged, the pellet was resuspended in isotonic KCl, and adjusted to an optical density of ~1.0 ± 0.05 at 578 nm (l = 0.5 cm), equivalent to ~2.8 × 10^9 viable cells/mL], and 2.5 mL of top agar, total volume = 3.85 mL. Procedures were essentially as described by Maron and Ames (*14*) and also for preparation of mammalian activation system (S9 mix) and mutagenicity testing. Toxicity was determined according to the method of Waleh et al. (*15*); the surviving fraction of bacteria was always >0.8 in measuring ranges. No measurements were performed in toxic ranges. Counting of colonies on plates for the *Salmonella*/reversion assay was performed by a Biotran II, automated colony counter (New Brunswick Scientific Co.).

Identification of Antimutagenic Compounds. Antimutagenic compounds, purified by preparative and repeated micropreparative HPLC from spinach, were controlled for purity by analytical HPLC. Pure compounds were identified by instrumental analyses.

Preparative, Micropreparative, and Analytical HPLC. *Preparative HPLC.* Thirty-six milliliters of solutions (possible solvents: water, methanol, water/methanol mixtures, 2-propanol, dichloromethane, and hexane), concentration up to 40 mg/mL, was applied (6 mL sample loop, six times) to a preparative YMC ODS-AQ column (50 mm × 260 mm, 10 μm particle size, Prochrom, Champigneulles, France). Gradient elution was carried out stepwise with water and methanol, each solvent containing 0.01% trifluoroacetic acid (TFA). Actual elution conditions were as follows: water for 0−5 min, water/methanol (80:20, v/v) for 5−12 min, water/methanol (70:30, v/v) for 12−20 min, water/methanol (60:40, v/v) for 20−40 min, water/methanol (40:60, v/v) for 40−45 min, and water/methanol (20:80, v/v) at ambient temperature and at a flow rate of 60−80 mL/min. The eluate was monitored with a photodiode array detector (DAD) at a wavelength of 315 nm. Fractions were collected according to the elution of peaks (see Figure 1), eventually concentrated, and further processed by micropreparative procedures.

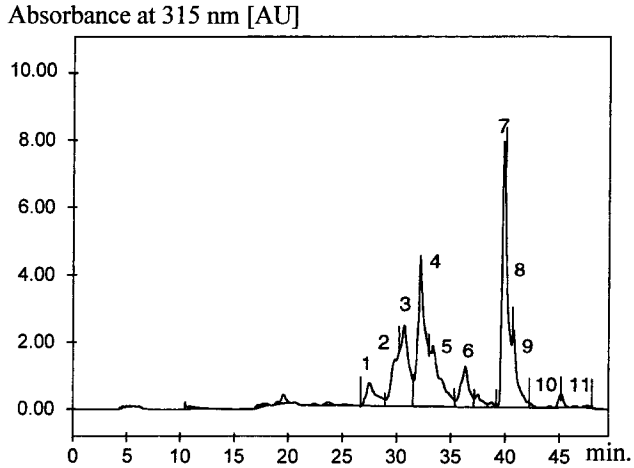

Figure 1. Preparative HPLC chromatogram of the methanol/water extract (70:30, v/v) from dry spinach (commercial product) after removal of lipophilic compounds by SPE. For a detailed description of the procedure, see Materials and Methods.

Micropreparative HPLC. Two hundred microliters of fractions, obtained by preparative HPLC, was applied (100 μL sample loop, two times) to a YMC ODS-AQ column (5 μm particle size, 4.6 mm × 250 mm). Gradient elutions were again carried out with methanol and water, containing 0.01% TFA, at ambient temperature at a flow rate of 1 mL/min; eluates were monitored by DAD at 315 nm. Elution conditions were as follows: micropreparative I, fractions 1 and 3 (Figure 1), methanol/water (75% water) for 0−2 min, methanol/water (75−70%) for 2−7 min, methanol/water (70%) for 7−8 min, methanol/water (70−60%) for 8−10 min, methanol/water (60%) for 10−12 min; micropreparative II, fractions 5 and 6, methanol/water (70% water) for 0−2 min, methanol/water (70−60%) for 2−5 min, methanol/water (60%) for 5−9 min; micropreparative III, fractions 8 and 9, methanol/water (65−55% water) for 0−7 min; micropreparative IV, fractions 10 and 11, methanol/water (50% water) for 0−3 min, methanol/water (50−40%) for 3−6 min, methanol/water (40%) for 6−8 min, methanol/water (40−20%) for 8−12 min. Fractions 2 and 4 of Figure 1 were not antimutagenic and were therefore not analyzed further; fraction 7 contained the pure compound **1**. It was necessary to perform up to 20 runs to obtain sufficient amounts (5−10 mg) of compounds to be characterized by antimutagenic potency and structure. Again, fractions were collected according to the elution of peaks and were analyzed for purity by analytical HPLC.

Analytical HPLC. An amount of 10−20 μL of a solution with flavonoids (glycosides, aglycons) or related compounds, dissolved in water, methanol/water, methanol, 2-propanol, or dichloromethane, was applied to a YMC ODS-AQ column (4.6 mm × 250 mm, 5 μm particle size) or a Bischoff ODS column (2.6 mm × 250 mm, 3 μm particle size). Solvents for elution were water and acetonitrile, both containing 0.01% TFA. Actual elution conditions were (analytical II) as follows: water for 0−10 min, gradient elution with 100−50% water for 10−40 min and 50−0% water for 40−50 min at a temperature of 30 °C and at a flow rate of 1 or 0.35 mL/min (second column). The eluate was monitored with a DAD at 260 and 315 nm. In later experiments, the isocratic elution phase could frequently be eliminated due to sufficient separation efficiency (analytical IIa). Other variations were as follows: analytical VI for methylated flavonoid aglycons, YMC ODS-AQ column (4.6 mm

× 250 mm, 5 μm particle size), elution gradient 100−50% water for 0−30 min and 50−0% water for 30−40 min, detection at 360 nm. All other conditions were as described under analytical II.

Characterization by Coupled Instrumental Procedures. For liquid chromatography−mass spectrometry (LC-MS) a YMC ODS-AQ microbore column (1 mm × 150 mm, 5 μm particle size) was utilized at ambient temperature and at a flow rate of 0.1 mL/min. Fractions from preparative HPLC were applied, and the conditions were as described under Micropreparative HPLC. UV−vis spectra were recorded, followed by mass spectra. However, mass spectra of only part of the compounds could be obtained because many flavonoid aglycons were eliminated in the particle beam interface. A thermospray interface with chemical ionization was, however, not available. For gas chromatography−mass spectrometry (GC-MS) flavonoid glycosides had to be hydrolyzed and aglycons had to be methylated (see below). Conditions of GC were as follows: 2 μL of the sample was injected to a silica capillary column, length = 15 m, diameter = 0.75 mm, with fused methylsiloxane, film diameter = 0.25 μm, gas (He) flow rate = 2 mL/min, temperature gradient from 100 to 280 °C, (0−2 min), 100 °C, (2−10 min), increase of 10 °C/min, (10−15 min) 180 °C, (15−25 min) an increase of 10 °C/min, and (25−30 min), 280 °C.

Hydrolysis of Glycosides and Methylation of Aglycons. For hydrolysis 5−10 mg of materials from fractions I, II, and IV was dissolved in 5 mL of water, and 0.4 mL of TFA was added. The mixture was refluxed for 60 min at 100 °C. After cooling, a brown precipitate was obtained, which was filtered and dried. Purity of compounds was checked by analytical HPLC procedure VI. Under these experimental conditions only flavonoid aglycons were present in all fractions. For methylation, flavonoids dissolved in acetone were treated with an ether solution of diazomethane until no more nitrogen was generated.

Ion Chromatography with a Pulsed Amperometric Detector (IC-PAD). Glycosides were detected electrochemically by applying a gold electrode. Conditions were as follows: measuring potential, 0.05 V; purification potentials, 0.60 and − 0.60 V; measuring time, 480 ms; purification times, 120 and 60 ms; range, 10 μA; display, positive; response time, 1 s. For chromatography of glycosides 20 μL of a solution was applied to a CarboPac PA 1 column (mono- and disaccharides) or to a CarboPac PA 100 column (oligosaccharides). Glycosides were eluted under isocratic conditions for 20 min with a mixture of 10 mM NaOH and 200 mM NaOH solution (3:1, v/v) at ambient temperature and at a flow rate of 0.8 mL/min. Identification of sugars was based on comparison of retention times with those of respective standards.

Instrumental Analyses. The compounds isolated by chromatography were analyzed by using the following procedures: The Fourier transform infrared (FT-IR) spectra (2 mg/250 mg of KBr) were determined with a Mattson Galaxy 2000 spectrometer and the UV−vis spectra with a UV-240 spectrometer (Shimadzu, Kyoto, Japan). Analytical and micropreparative HPLC was performed with a Hewlett-Packard liquid chromatograph (Agilent Technologies, Waldbronn, Germany). For preparative HPLC two high-pressure pumps (HD 2-200; Besta, Wilhelmsfeld, Germany) and a UV detector (Lambda 1000; Bischoff, Leonberg, Germany) were used. For LC-MS a Waters integrity system (Waters, Milford, MA) with a particle beam interface was used. For gas chromatography−mass spectrometry (GC-MS) a Varian GC 3400 (Varian, Walnut Creek, CA) and a Magnum ITD (Finnigan, Bremen, Germany) were utilized. Electron impact mass spectra (EI-MS) as well as fast atom bombardment mass spectra (FAB-MS) were obtained on a Finnigan-Mat 8200. For FAB-MS compounds were dissolved in DMSO, whereas glycerol was used as the matrix. 1D proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear resonance (^{13}C NMR) spectra as well as 2D ^{1}H,^{1}H-COSY, and ^{1}H,^{13}C-COSY spectra were recorded on a Bruker AM 250 spectrometer. ^{1}H NMR spectra were performed of all compounds isolated (sample size = 50 μg−2 mg) and of various fractions containing a maximum of three compounds. A ^{13}C NMR spectrum could be recorded only with compound **1** due to the amounts necessary (20−30 mg). All work was performed in a glovebox under an atmosphere of argon because most isolated compounds were hygroscopic. Samples were dissolved in hexadeuteriodimethyl sulfoxide (DMSO-d_6) and were later recovered by solid-phase extraction (SPE) using ODS-reversed phase material, 50 μm (Bakerbond), equilibrated with water (elution of flavonoids with methanol). For analyses of glycosides a DX-300 (Dionex, Idstein, Germany) was used.

RESULTS

Purification of the Antimutagens in Spinach. Extraction of a commercial product of dry spinach (100 g) with the solvent sequence *n*-hexane, dichloromethane, 2-propanol, and methanol/water (50:50, v/v) resulted in the isolation of 0.37, 0.65, 1.2, and 5.3 g of materials, respectively (total yield = 7.52 g). The juice as well as the water insoluble compounds extracted from the residues in these and in preceding investigations exerted strong antimutagenicity against 2-amino-3-methylimidazo[4,5-*f*]quinoline (*11*, *12*). It was already known that besides chlorophyll, spinach probably contains many antimutagens including acidic, basic, and neutral compounds (*12*), so in the present study we analyzed only materials extracted with methanol/water (70:30, v/v) from 108 g of dry spinach in order to identify polar antimutagens. The dried residue of this extract, freed from nonpolar compounds such as chlorophyll(s) and carotenoids by SPE, was dissolved in water and subjected to preparative HPLC. The results are shown in Figure 1.

Identification of the Isolated Antimutagenic Compounds. When fraction 7 of preparative HPLC was subjected to micropreparative HPLC according to procedure III, a single antimutagenic compound with a purity of >95%, designated compound **1**, was detected. Application of the same procedure to the combined fractions 8 and 9 resulted in the separation of compounds **1**, **2**, and **3** with retention times of 7.46, 8.24, and 8.64 min, respectively. Analytical HPLC (procedure IIa) proved that these peaks comprised pure compounds with retention times of 26.0, 27.0, and 27.4 min.

The FT-IR spectrum of compound **1** in KBr showed the presence of an aromatic keto group (ν_{max} = 1550−1650 cm^{-1}), a COOH group (ν_{max} = 1700 cm^{-1}), and at least one phenolic OH function (ν_{max} = 3000 cm^{-1}). An additional broad absorption band at 3600 cm^{-1} could be related to OH vibrations with a typical line broadening due to intra- and intermolecular hydrogen bonding. The EI/MS spectrum showed a base peak at *m/z* 344 (A^+, molecular ion of the aglycon $[C_{17}H_{12}O_8]^+$, see Figure 2) and major fragment ion peaks at *m/z* 343 ($A^+ - 1$), 301, and 180 as well as minor ion peaks at *m/z* 314, 280, 271, and 207. A molecular ion peak of the flavonol glucuronide could not be observed in accordance with the expectation that a cleavage of the O-glycosidic bond occurred, whereas a C-glycosidic bond would have survived. The absence of any signal at M^+ is therefore indicative of an *O*-glucuronide. However, the glucuronic acid has a molecular mass of 177 and a corresponding weak signal could be detected. The ^{13}C NMR spectrum showed signals (see Figure 2 for numbering) at δ (ppm) 151.6 (C-2), 129.0 (C-3), 179.2 (C-4), 153.8 (C-5), 146.8 (C-6), 147.6 (C-7), 89.9 (C-8), 155.1 (C-9), 107.6 (C-10), 123.8 (C-1′), 117.6 (C-2′), 140.3 (C-3′), 138.8 (C-4′), 116.5 (C-5′), 119.7 (C-6′), 75.8 (1″), 72.9 (2″), 75.8 (3″), 71.3

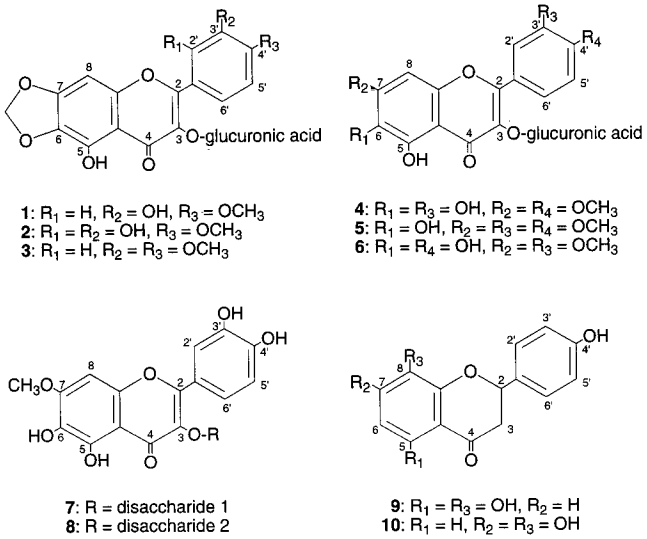

Figure 2. Chemical structures of antimutagenic flavonoids isolated from spinach.

(4″), 75.8 (5″), 170.3 (6″), 138.8 (OCH_3), and 102.6 (OCH_2O). This established the presence of 12 quaternary, 9 tertiary, 1 secondary, and 1 primary carbon atom. The ^{1}H NMR spectrum (DMSO-d_6) gave signals at δ 6.90 (1H, s, 8-H), 7.53 (1H, d, ^4J = 2.5 Hz, 2′-H), 7.17 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.48 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.70 (1H, s, 5-OH), 9.2 (1H, s, 3′-OH), 3.80 (3H, s, 4′-OCH_3), 6.15 (2H, s, $-OCH_2O$), 5.00 (1H, d, ^3J = 7.5 Hz, 1″), 3.90 (1H, d, ^3J = 9.5 Hz, 5″), 3.30−3.50 (other sugar protons). The coupling constant of the proton at 1″ indicated a β-linkage (β-anomer, J = 7−8 Hz; α-anomer, J < 7 Hz). The identity of the glycosidic acid could be determined from the NMR data because only galacturonic or glucuronic acid could be expected. Galacturonic acid possesses an equatorially linked H atom at C-4, whereas glucuronic acid possesses an axially linked proton. Both acids possess an equatorially linked H atom at C-5. The vicinal coupling J_{aa} shows a value of >7 Hz. Because the H at G-5 of ^{1}H NMR indicated J = 9.5 Hz, the galacturonic acid could be excluded. During chromatography in acetonitrile/water [0.01% TFA; 50:50 (v/v)] compound **1** showed absorption bands with λ_{max} of 209, 253, 280, and 350 nm, which are typical for flavonoids. Under IC-PAD conditions a peak with a retention time (t_R) of 2.66 min was observed, identical with the reference compound glucuronic acid. These data agree with the assumption that compound **1** in spinach was identical with 5,3′-dihydroxy-4′-methoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (Figure 2).

On the basis of FT-IR, UV−vis, and ^{1}H NMR spectroscopy, compound **2** in spinach was similarly identified as 5,2′,3′-trihydroxy-4′-methoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (Figure 2). The FT-IR spectrum, which was very similar to that of compound **1**, indicated the presence of an aromatic carbonyl function, a carboxylic acid group, and phenolic OH group(s). The ^{1}H NMR spectrum showed signals at δ 6.95 (1H, s, 8-H), 7.20 (1H, d, ^3J = 9.5 Hz, 5′-H), 8.02 (1H, d, ^3J = 9.5 Hz, 6′-H), 12.70 (1H, s, 5-OH), 9.20 (2H, s, 2′-OH and 3′-OH), 3.80 (3H, s, 4′-OCH_3), 6.15 (2H, s, OCH_2O), 5.15 (1H, d, ^3J = 7.5 Hz, 1″), 3.90 (1H, d, ^3J = 9.5 Hz, 5″), 3.30−3.50 (other sugar protons). Compound **2** showed UV−vis absorption bands with λ_{max} of 219, 278, and 335 nm.

In the same way, compound **3** in spinach was identified as 5-hydroxy-3′,4′-dimethoxy-6,7-methylenedioxyflavonol 3-*O*-β-glucuronide (Figure 2). Again, the FT-IR spectrum was closely related to that of compound **1**. The EI/MS spectrum gave a base peak at m/z 358 (A^+), major fragment ion peaks at m/z 343 ($A - CH_3$) and 315, and minor signals at m/z 327 and 280. ^{1}H NMR showed signals at δ 6.98 (1H, s, 8-H), 7.65 (1H, d, ^4J = 2.5 Hz, 2′-H), 7.35 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.64 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.60 (1H, s, 5-OH), 3.70 (3H, s, 3′-OCH_3), 3.80 (3H, s, 4′-OCH_3), 6.15 (2H, s, OCH_2O-), 5.25 (1H, d, ^3J = 7.5 Hz, 1″), 3.90 (1H, d, ^3J = 9.5 Hz, 5″), 3.30−3.50 (other sugar protons). During chromatography compound **3** showed absorption bands with λ_{max} of 216, 250, 277, and 340 nm, typical for flavonoids.

Fraction 6 of preparative HPLC (see Figure 1) was subsequently subjected to micropreparative HPLC according to procedure II. A series of peaks was detected, among them two major peaks with t_R of 7.37 and 8.00 min, which comprised compounds with antimutagenic activity against 2-amino-3-methylimidazo[4,5-*f*]quinoline. Analytical HPLC, procedure VI, proved that these peaks comprised pure compounds with t_R of 13.66 and 14.75 min, designated compounds **4** and **5**.

After instrumental analysis, compound **4** from spinach was assigned to be 5,6,3′-trihydroxy-7,4′-dimethoxyflavonol 3-*O*-β-glucuronide (Figure 2). The EI/MS spectrum of compound **4** gave a base peak at m/z 346 (A^+), major fragment ions with m/z 345, 331, 328, and 303, and minor signals at m/z 289, 273, 260, 232, 207, 194, 177 (M^+ of glucuronic acid), and 164. The ^{1}H NMR spectrum showed signals at δ 6.51 (1H, s, 8-H), 7.51 (1H, d, ^4J = 2.5 Hz, 2′-H), 7.15 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.47 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.60 (1H, s, 5-OH), 9.20 (1H, s, 3′-OH), 3.80 (3H, s, 4′-OCH_3), 10.80 (1H, s, 6-OH), 3.75 (3H, s, 7-OCH_3), 5.05 (1H, d, ^3J = 7.5 Hz, 1″), 3.90 (1H, d, ^3J = 9.5 Hz, 5″), 3.30−3.50 (other protons of glucuronic acid). Compound **4** showed absorption bands with λ_{max} of 208, 252, 270, and 340 nm, again characteristic for flavonoids.

Again, compound **5** from spinach was identified as 5,6-dihydroxy-7,3′,4′-trimethoxyflavonol 3-*O*-β-glucuronide (Figure 2). The FT-IR spectrum indicated the presence of an aromatic carbonyl function, a carboxylic acid group, and a phenolic OH group. The ^{1}H NMR spectrum showed signals at δ 6.56 (1H, s, 8-H), 7.62 (1H, d, ^4J = 2.5 Hz, 2′-H), 7.25 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.58 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.60 (1H, s, 5-OH), 3.85 (3H, s, 3′-OCH_3), 3.80 (3H, s, 4′-OCH_3), 10.80 (1H, s, 6-OH), 3.75 (3H, s, 7-OCH_3), 5.05 (1H, d, ^3J = 7.5 Hz, 1″), 3.90 (1H, d, ^3J = 9.5 Hz, 5″), 3.30−3.50 (other protons of glucuronic acid). Compound **5** showed absorption bands with λ_{max} of 208, 253, 270, and 340 nm, nearly identical with those of the spectrum of compound **4**.

When fraction 1 (Figure 1) obtained by preparative HPLC was subjected to micropreparative HPLC, according to procedure I, four major peaks representing compounds with antimutagenic activities against 2-amino-3-methylimidazo[4,5-*f*]quinoline were observed, with t_R values of 3.98 min (compound **7**), 4.97 min (compounds **6** and **8**), 6.36 min (compound **9**), and 6.89 min (compound **10**). Under the analytical HPLC conditions, procedure II, t_R values of 17.15, 17.99, and 18.23 min were observed for the pure compounds **7**, **8**, and **6**, respectively. After instrumental analysis, compound **6**

was identified as a 5,6,4′-trihydroxy-7,3′-dimethoxyflavonol 3-*O*-disaccharide (Figure 2). The ^{1}H NMR spectrum (in DMSO-d_6) indicated signals at δ 6.55 (1H, s, 8-H), 7.90 (1H, d, ^4J = 2.5 Hz, 2′-H), 6.90 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.46 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.70 (1H, s, 5-OH), 3.85 (3H, s, 3′-OCH$_3$), 9.80 (1H, s, 4′-OH), 10.75 (1H, s, 6-OH), 3.75 (3H, s, 7-OCH$_3$), 5.50 (1H, d, ^3J = 7.5 Hz, 1″), 4.10 (1H, d, ^3J = 9.5 Hz, 2″), 3.30−3.50 (other sugar protons). During chromatography in acetonitrile/water [0.01% TFA; 50:50 (v/v)] compound **6** showed absorption bands with λ_{max} of 205, 255, 269, and 351 nm, again typical for a flavonoid. When compound **6** was hydrolyzed and the sugars were analyzed by IC-PAD, three peaks were observed: a major peak with t_R of 5.48 min and two additional peaks with t_R of 9.98 and 12.72 min. The first peak was identical with glucose; the two other peaks, however, could not be attributed unambiguously to known glycosides. Under the conditions of hydrolysis used, confirmed by control experiments, only monosaccharides were generated. Measurements with FAB-MS did not result in the detection of a molecular ion peak, whereas the number of sugar protons as deduced from the ^{1}H NMR spectrum identified the original sugar substituent as a disaccharide.

Compounds **7** and **8** were identified as 5,6,3′,4′-tetrahydroxy-7-methoxyflavonol 3-*O*-disaccharides (Figure 2). The EI/MS spectrum of compound **7** (and **8**) gave a base peak at *m/z* 332 (A^+) and major fragment ions with *m/z* 314, 303, and 289. The latter signal with $M^+ - 43$ is typical for a ketone possessing an aryl-OCH$_3$ function. The ^{1}H NMR spectrum of compound **7** showed signals at δ 6.44 (1H, s, 8-H), 7.50 (1H, d, ^4J = 2.5 Hz, 2′-H), 6.78 (1H, d, ^3J = 9.5 Hz, 5′-H), 7.52 (1H, q, ^3J = 9.5 Hz, ^4J = 2.5 Hz, 6′-H), 12.70 (1H, s, 5-OH), 9.20 (1H, s, 3′-OH), 9.80 (1H, s, 4′-OH), 10.75 (1H, s, 6-OH), 3.75 (3H, s, 7-OCH$_3$), 5.35 (1H, d, ^3J = 7.5 Hz, 1″), 4.00 (1H, d, ^3J = 9.5 Hz, 2″), 2.80−3.70 (other sugar protons). The ^{1}H NMR spectrum of compound **8** was identical with that of compound **7** except for the signals at 7.62 (1H, q, 6′-H) and 7.53 (1H, d, 2′-H). This difference can be caused only by the glycosidic substituent at C-3. This substituent could not be identified unambiguously, for the same reasons as outlined for compound **6**. Again, there were no differences with respect to the FT-IR and UV−vis spectra. Compounds **7** and **8** showed absorption bands with λ_{max} of 207, 259, 269, and 346 nm.

Fraction 1 from preparative HPLC (Figure 1) comprised two additional compounds with antimutagenic activities, designated compounds **9** and **10,** according to micropreparative HPLC, procedure I (t_R of 6.36 and 6.89 min). Analytical HPLC, procedure VI, showed a t_R of 11.18 min for compound **9** and a t_R of 11.49 min for compound **10**. The ^{1}H NMR spectrum and UV−vis spectrum of compound **9** were identical to those of 5,8,4′-trihydroxyflavanone (*16*) (Figure 2), whereas the respective spectral data of compound **10** were equivalent to those of 7,8,4′-trihydroxyflavanone (*17*) (Figure 2).

When fraction 3 of Figure 1, obtained by preparative HPLC, was subjected to micropreparative HPLC, according to procedure I, 4 major and at least 11 minor peaks were observed, but only 1 peak, t_R of 10.09 min, contained a compound, designated compound **11**, with antimutagenic activity. Again, the same procedure, this time HPLC performed according to procedure II, resulted in the detection of two major peaks, t_R of 6.74 min (compound **13**) and t_R of 7.36 min (compound **12**), with antimutagenic activities and at least 15 additional but inactive minor peaks. Analytical HPLC, procedure VI, indicated the following t_R values: compound **11**, 12.19 min; compound **12**, 13.27 min; and compound **13**, 12.75 min.

Compounds **11**−**13** were available in purities of >90% for instrumental analysis. The ^{1}H NMR spectrum of compound **11** in DMSO-d_6 gave the following signals: 7.56 (1H, s), 7.50 (1H, d, ^3J = 9.5 Hz), 7.45 (1H, d, ^3J = 18 Hz), 7.35 (1H, d, ^3J = 9.5 Hz), 7.10 (1H, d, ^3J = 9.5 Hz), 6.85 (1H, d, ^3J = 9.5 Hz), 6.80 (1H, d, ^3J = 9.5 Hz), 6.48 (1H, s), 6.25 (1H, d, ^3J = 18 Hz), 12.80 (1H, s, OH), 10.70 (1H, s, OH), 9.60 (1H, s, OH), 9.20 (1H, s, OH), 9.70 (1H, s, OH), 3.75 (3H, s, OCH$_3$), 3.90 (3H, s, OCH$_3$). In addition, signals between 3.8 and 2.10 were detected, which were attributed to sugar protons. Compound **11** showed absorption bands with λ_{max} of 212, 251, 272, 304 (shoulder), and 334 nm. The ^{1}H NMR spectrum of compound **12** showed the following signals: 7.90 (1H, s), 7.50 (1H, d, ^3J = 9.5 Hz), 7.50 (1H, d, ^3J = 18 Hz), 7.35 (1H, d, ^3J = 9.5 Hz), 7.10 (1H, d, ^3J = 9.5 Hz), 6.85 (1H, d, ^3J = 9.5 Hz), 6.85 (1H, d, ^3J = 9.5 Hz), 6.58 (1H, s), 6.20 (1H, d, ^3J = 18 Hz), 12.80 (1H, s, OH), 10.70 (1H, s, OH), 9.85 (1H, s, OH), 9.65 (1H, s, OH), 3.90 (3H, s, OCH$_3$), 3.85 (3H, s, OCH$_3$), 3.70 (3H, s, OCH$_3$). In addition, signals between 3.8 and 2.10 were detected that were attributed to sugar protons. Compound **12** showed absorption bands with λ_{max} of 212, 249, 273, 298 (shoulder), and 333 nm. The ^{1}H NMR spectrum of compound **13** differed from that of compound **11** only with respect to a signal at 9.7 (s, OH), which was absent in the spectrum of this compound. Compound **13** showed absorption bands with λ_{max} of 209, 258, 275, 316, and 360 (shoulder) nm. The UV−vis spectra of compounds **11**−**13** indicated a flavonoid structural type. Assignment to a known parent compound (aglycon) was, however, not possible due to the complexity of the chemical shifts and couplings as well as the high number of aromatic H (9 H), OH (4−5), and OCH$_3$ (2−3) groups present. Compounds **11** and **12**, however, differ only in the way that one hydroxyl group of compound **11** has been methylated in compound **12**, whereas compound **13** has one hydroxyl function less than compound **11**. All three compounds contain a sugar moiety the structure of which could not be elucidated due to the small amounts of isolated materials. Nevertheless, according to ^{1}H NMR spectra a glucose moiety is present and the coupling constant J < 7.4 Hz of the proton at 1″ may indicate a β-glycosidic bonding to the aglycon.

DISCUSSION

Spinach is devoid of flavonoids widespread in most plants, such as kaempferol, quercetin, myricetin, apigenin, and luteolin, but contains unique compounds. These are patuletin (quercetagetin 6-methyl ether) and spinacetin (quercetagetin 6,3′-dimethyl ether) (*18*), a glycoside with an aglycon tentatively identified as 3-methoxy-6,7-methylenedioxyquercetagetin (*19*), spinatoside, 3,6-dimethylquercetagetin 4′-*O*-glucuronide (*20*), the 4′-glucuronides of 5,7,4′-trihydroxy-3,6,3′-trimethoxyflavone, 5,3′,4′-trihydroxy-3-methoxy-6,7-methylenedioxyflavone, and 5,4′-dihydroxy-3,3′-dimethoxy-6,7-methylenedioxyflavone (*21*), and patuletin 3-gentiobioside, patuletin 3-glucosyl-(1→6)-[apiosyl-(1→2)]-glucoside, and spinacetin 3-gentiobioside (*22*). In our own investigations we have identified three so far unknown

Table 1. Antimutagenic Potencies of Compounds Isolated from Spinach (*S. oleracea*) on Mutagenicity Induced by 2-Amino-3-methylimidazo[4,5-*f*]quinoline in *S. typhimurium* TA 98

compd	yield (mg/100 g)	IC_{50}[a] (nmol/mL of top agar)	maximum[b] reduction of mutagenicity	aglycon[c] of compd	IC_{50}[a] (nmol/mL of top agar)	maximum[b] reduction of mutagenicity
1	300	49.9	79.0	**1**	54.0	73.7
2	59	38.7	80.0	**2**	40.1	77.3
3	21	58.2	64.7	**3**	53.7	70.1
4	69	44.7	79.2	**4**	47.7	74.2
5	21	24.2	79.1	**5**	24.8	77.1
6	17		38.7	**6**	10.4	79.6
7	56		34.3	**7**	13.0	65.0
8	70		39.5	**8**	13.0	70.1
9	45	not reached	21.6[d]			
10	45	not reached	31.2[d]			
11	33		40.0	**11**		75.4
12	21		49.1	**12**		69.5
13	10.5		21.8	**13**		59.1

[a] IC_{50} is the concentration of a flavonoid in nmol/mL of top agar (μM) required to inhibit the mutagenic activity by 50%, calculated from corresponding dose–response curves. [b] Maximum concentration tested was 77.9 μg/mL of top agar unless otherwise indicated. [c] After chemical hydrolysis. [d] Maximum concentration was 93.5 μg/mL.

6,7-methylenedioxyflavonol glucuronides (compounds **1**–**3**) with hydroxyl and/or methoxyl groups at C-5, C-2′, C-3′, and C-4′. These compounds fit into the group of methylenedioxyflavonoids with substitution at C-6/C-7 and/or at C-3′/C-4′ (*23*). Two other flavonol glucuronides (compounds **4** and **5**) described here for the first time were methyl ether derivatives of quercetagetin (3,5,6,7,3′,4′-hexahydroxyflavone) and may be formally generated by opening of the five-membered dioxolane ring system at C-6/C-7 from compounds **1** and **3**. In spinach samples investigated by us, the glucuronide moiety was always linked to the hydroxyl group at C-3, in contrast to other researchers who had described various 4′-glucuronides (*24*). Identification of flavonol glycosides by us agreed in part with the results of Aritomi et al. (*21*). With respect to sugars we detected only disaccharides, comprising glucose and two unidentified glycosides, whereas ref *21* identified the disaccharide gentiobiose and a new trisaccharide. Gentiobiose with two glucose molecules 1→6 linked might well be one of our incompletely characterized disaccharides. In general, the basic oxygenated structure pattern of quercetagetin was detected by all groups and the 6,7-methylenedioxy derivative by most investigators as well as the existence of glucuronides and 3-hydroxy linked saccharides. There is, however, major disagreement with respect to methylation of hydroxyls of quercetagetin and 6,7-methylenedioxyquercetagetin between us and others. In addition, various flavonoids of so far unknown structure seem to exist in spinach (*19*, *22*, this investigation). In our own investigations, we detected glycosides with aglycons which resemble typical flavonoids but must comprise more complicated systems with a total of six to seven hydroxyl and methoxy groups. Finally, two flavonoids present as aglycons were identified as well-known flavanones: 5,8,4′-trihydroxyflavanone (compound **9**) (*16*) and 7,8,4′-trihydroxyflavanone (compound **10**) (*17*).

The aim of this research was to isolate and characterize from spinach compounds with antimutagenic activities against 2-amino-3-methylimidazo[4,5-*f*]quinoline. Antimutagenic potencies of flavonoids detected here were compared with those of a series of 64 flavonoids investigated previously (*13*). It was confirmed that glycosides, among them flavonol 3-glycosides, were less potent than the corresponding aglycons (compounds **6**–**8** and **11**–**13**). Because various flavonoid glycosides in contrast to the respective aglycons did not exert any antimutagenicity, additional glycosides may exist in spinach but have remained undetected in these investigations. The subgroup of flavonol 3-glucuronides, however, did not differ from their aglycons in antimutagenic potency (compounds **1**–**5**, Table 1). The 6,7-methylenedioxy function in quercetagetin derivatives seems to have no influence on antimutagenicity (compounds **1**–**3** versus **4** and **5**). The introduction of a hydroxyl/methoxy function at C-6, however, considerably reduced antimutagenic potency: IC_{50} values of flavonol, 6-methoxyflavonol, and quercetin (3,5,7,3′,4′-OH) were 0.67, 4.0, and 3.2 μM, respectively, whereas quercetagetin with an additional 6-OH function was inactive. Therefore, patuletin will probably lack antimutagenicity against this imidazoquinoline as well as spinacetin glycosides and may not be detectable in our test system. However, a total of four mono-, di-, and trimethyl ethers of quercetagetin exerted antimutagenicity, the potencies of which (IC_{50} = 25–55 μM) were somewhat lower than those of morin, robinetin, and myricetin (3,5,7,3′,4′,5′-OH). In addition, due to the inactivity of quercetagetin, its 6,7-methylenedioxy derivative and their glucuronides, if present, would not be detected. Flavanones with an 8-hydroxyl group were not available as reference compounds for antimutagenicity testing. However, such a hydroxyl group may reduce antimutagenic potency considerably because flavanones with a 5,7,4′-hydroxyl substitution pattern (naringenin) and related compounds exerted strong antimutagenicity against heterocyclic amines.

By means of activity-linked enrichment of antimutagenic compounds from nutritional and medicinal plants, applying the same procedures as in these investigations, various flavonoids have been isolated and identified. The flavonoids luteolin, galangin, and quercetin were isolated from peppermint (*Mentha piperita*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) (*25*), and oregano (*Origanum vulgare*) (*26*), respectively, and, in addition, cirsimaritin and salvigenin from rosemary (*Rosmarinus officinalis*) (*27*) as well as genkwanin, cirsimaritin, hispidulin, and apigenin from carqueja (*Baccharis trimera*) (*28*), a Brazilian folk medicine plant, and were shown to exert antimutagenicity against 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole. 3-Kaempferyl *p*-coumarate from bay laurel (*Laurus nobilis*), however, was a novel compound (*29*). In previous investigations of plant extracts from fruits and vegetables, we showed that spinach juice, as well as

various solvent extracts from residues, exerted especially strong antimutagenic effects against 2-amino-3-methylimidazo[4,5-*f*]quinoline (*11*, *12*). [Strong protective effects of spinach were also exerted against in vivo clastogenicity of benzo[*a*]pyrene and cyclophosphamide in mice (*8*).] In the residues, a series of active compounds of unknown structure were present, among them basic and acidic substances, but at that time chlorophyll was the only antimutagen identified. Within the limits of experimental error, ~50−100% of the total antimutagenicity of original spinach juice ($IC_{50} = 8.4 \pm 4.1$ μL) (*11*) can be explained by the presence of the flavonol glucuronides, compounds **1**−**6** (525 mg/100 g of spinach; IC_{50} = 50 μM; 4.6 g of solids/100 mL of spinach juice) (*30*).

ACKNOWLEDGMENT

Our thanks and appreciation are given to H. Kohlshorn, Mainz, for helpful discussions regarding the identification of flavonoids.

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Received for review November 15, 2000. Revised manuscript received March 22, 2001. Accepted March 23, 2001. We are grateful for financial support to Bestfoods Europe, Heilbronn, Germany. In addition, G.K. was personally supported by the Deutsche Forschungsgemeinschaft (Grant SFB 302/A10) during part of this work.

JF0013712

EXHIBIT F

Eur J Nutr (2004) 43 : 15–22
DOI 10.1007/s00394-004-0434-x

ORIGINAL CONTRIBUTION

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Kinetics of gastro-intestinal transit and carotenoid absorption and disposal in ileostomy volunteers fed spinach meals

Summary *Background* Reports of low carotenoid absorption from food sources has undermined their postulated 'protective' role as one of the active agents in diets rich in vegetable matter. *Aims of the study* This study quantified β-carotene and lutein absorption from a representative green vegetable with different degrees of processing, using both mass balance and metabolic modelling of triglyceride-rich lipoprotein plasma fraction (TRL) response. *Method* Whole or chopped-leaf cooked spinach was fed to volunteers (n = 7, paired) with vegetable oil (40 g) in yoghurt. Blood and ileal effluent samples were collected for up to 24 h. Effluent and TRL samples were analysed for lutein and β-carotene by HPLC. A digesta transit model was used to describe meal transit and a single compartment model used to predict percentage absorption from the plasma TRL response. *Results* Mass balance showed 25 % of lutein and β-carotene were absorbed from chopped spinach, compared with 25 % β-carotene and 40 % lutein from whole-leaf spinach. Increased lutein absorption correlated to slower gastrointestinal (GI) transit for the whole-leaf meal. An area under the curve (AUC) response for the TRL fraction, found in 50 % of cases, was not confined to those with the greatest percentage absorption. Absorption by mass balance and TRL AUC indicate a half-life of newly absorbed carotenoid around 11 min *Conclusion* GI residence time appears to have an effect on the absorption of lutein but not β-carotene. Rapid clearance is probably the main reason for absence of measurable plasma concentration excursions. Lack of plasma response cannot be interpreted as lack of carotenoid absorption without knowledge of the absorption and disposal kinetics.

Key words Lutein – β-carotene – absorption – ileostomy – model

Received: 21 October 2002
Accepted: 26 May 2003
Published online: 6 January 2004

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Abbreviations

AUC	Area under the curve
BMI	Body mass index
GI	Gastro-intestinal
HDL	High-density lipoproteins
HPLC	High performance liquid chromatography
LDL	Low-density lipoproteins
SD	Standard deviation
SI	Small intestine
TGWM	Total gastrointestinal washout method
TRL	Triglyceride rich lipoproteins
VLDL	Very low density lipoproteins
IV	Intravenous

Introduction

The absorption of carotenoids from test meals is reported to range between 2–90 % depending upon the test material, the model used to assess absorption and interpretation of data obtained [1]. Reports of low absorption values [2, 3], obtained using changes in plasma concentration have undermined the hypothesis that the carotenoids are one of the active protective constituents of diets high in vegetables and fruits, and that supplemental green vegetable feeding may be ineffective in relieving retinol deficiency [4]. Plasma concentrations of the carotenoids may be elevated significantly in chronic dosing studies [5, 6] but the absolute amount absorbed cannot be quantified by this approach.

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The absorption of carotenoids has been attempted in human volunteers by a number of methods. Oral-faecal mass balance [7], whole plasma response after chronic [3, 8–10,] or acute [11, 12] dosing, triglyceride-rich lipoprotein (TRL) response after an acute dose [2, 13], radioactive tracers [14, 15], stable isotope tracers [16–18], total gastrointestinal washout, mass balance method (TGWM) [19] and the ileostomy mass balance model [20].

Of the mass balance methods, the oral-faecal approach has been the most commonly used. However, the method is prone to losses of carotenoid through their exposure to the microflora of the large intestine and precise faecal collection may be a problem. Estimates of absorption by this method are therefore predicted to be high and variable.

A second, frequently used approach is measuring carotenoid concentration in whole plasma following an acute (single meal), or chronic, dose (multiple meals over several days/weeks). Plasma response after a single meal has identified many individuals who do not appear to 'respond', i. e. no measurable change in plasma concentration; sometimes interpreted as no, or low, carotenoid absorption. This interpretation has been challenged following consideration of plasma clearance kinetics and the likelihood, or not, of observing a perturbation in the plasma pool after a single meal [20]. In chronic feeding studies, plasma concentrations of carotenoids are more likely to be significantly elevated, but the amount absorbed still cannot be quantified without a knowledge of the clearance kinetics from all appropriate plasma pools and re-exportation kinetics from liver and other tissues. Furthermore, changes in plasma concentration after feeding a 'standard' isolated compound vs. a food are usually assumed to follow a linear dose response. At best, absorption from the food can only be expressed as a percentage of the absorption from the 'standard' dose.

In the present study the ileostomy approach has been selected as the preferred mass balance method for obtaining quantitative data on the absorption of carotenoids from an important food source (green leafy vegetable). Whilst there may be some microbial activity in the SI through colonisation of the terminal ileum, appropriate collection and storage of effluent minimises the potential confounding influence of microbial action, and variable residence time in the large bowel.

As an alternative to mass balance measurements, the study described here also included carotenoid analysis of whole plasma and the triglyceride-rich lipoproteins to produce comparative data between loss of carotenoid from ileal effluent and appearance of carotenoids in blood. Newly absorbed carotenoids, from a meal given after an overnight fast, appear in the TRL. The AUC for the TRL-carotenoid response is measured, clearance rate assumed to be the same as for chylomicrons and carotenoid absorption quantified [13]. However, the actual clearance kinetics for chylomicron remnant half life [21–23], or chylomicron triacylglycerol half life are not known for the individual volunteers, and have to be assumed from limited published information [24], gives rise to large differences in carotenoid absorption values, depending on the half life value selected. By quantifying the amount of carotenoid lost in ileal effluent, together with the size of the TRL excursion, calculation of the carotenoid half-life was possible and assumptions relating to chylomicron remnant half-life tested.

In summary, the objectives of the study were to quantify β-carotene and lutein absorption from a representative green vegetable with different degrees of processing; using both mass balance and metabolic modelling of TRL response. This dual approach allowed comparison of the data sets obtained and testing of assumptions relating to clearance kinetics of carotenoids from the TRL fraction.

Experimental

A group of seven ileostomy volunteers who had minimal ileal resection (< 15 cm) for ulcerative colitis (5 women and 2 men), mean (SD) age 51 (7.6) y, weight 80.1 (16.4) kg gave informed written consent to the study which was approved by the Norwich District Ethics Committee. Volunteers had BMI values of 19–27, fasting plasma cholesterol ≤ 6.5 mmol/l, fasting plasma triglycerides ≤ 2.3 mmol/l, plasma β-carotene 0.1–1.0 μmol/l and plasma retinol ≥ 0.1 μmol/l, were non-smokers, not taking medication or dietary supplements. All but one consented to provide serial blood samples over the duration of the 2 study periods. Volunteers attended the Human Nutrition Unit on two occasions, at least 6 weeks apart, having avoided excessive carotenoid intake (a list of foods was provided) for 24 h before the study day. They arrived at 08.00 h having fasted from 19.00 h the previous evening and having performed their usual morning routines.

Volunteers were cannulated (antecubital/cephalic), provided a baseline fasting blood sample (20 ml), emptied their appliances (baseline effluent collection) and were then given approximately 150 g of either cooked whole, or cooked finely chopped, leaf spinach prepared from the same harvest. The spinach meal contained approximately 15 mg lutein and 10 mg β-carotene. The spinach, which had been blanched, drained, frozen and stored at –40 °C in heat sealed foil laminate pouches, was reheated in boiling water to a core temperature in excess of 72 °C for 3 min. A sub-sample of the spinach meal was retained at –70 °C for analysis. The spinach meal was followed by 400 g of skimmed milk yoghurt containing 40 g of low vitamin E sunflower oil, 20 g of sucrose and chocolate flavouring. The study was timed from when

the spinach was consumed (t = 0). Defined carotenoid-free midday (t = 4.5h) and evening (t = 10 h) meals were provided and carotenoid free drinks were freely available at all times. The midday meal provided 20 g of fat (25 % of energy) and the evening meal 42 g of fat (28 % of energy).

Volunteers remained seated in an armchair for the duration of the study except for toilet visits. This procedure was adopted to harmonise physical activity in all volunteers although it is recognised that it may have had an impact on gut motility. Blood samples (20 ml) were drawn every 2 h from t = 0 up to 12 h, into lithium heparin blood tubes, centrifuged, the plasma separated and frozen at –70 °C before further treatment. Total ileal effluent was collected every 2 h up to 12 h and then as discrete timed samples up to 24 h at the volunteers' own convenience. All effluent samples and retained food samples, collected into polythene bags, were spread into thin sheets within the bag, frozen on solid CO_2, weighed and stored at –70 °C. Plasma (5 ml) was density adjusted with potassium bromide, layered into saline (1.006 sg) and ultracentrifuged for 4 h at 64,000 g [25] to separate the lipoproteins into TRL, LDL, and HDL fractions. The fractions were then aspirated from the centrifuge tube and stored at –70 °C.

Plasma and plasma fractions were extracted using hexane and the carotenoid content assayed by HPLC [6] with a limit of quantification < 1 ng. Effluent and spinach samples (ca. 8 g) were broken from the frozen sheets, placed in 50 ml screw top glass centrifuge tubes and 20 ml of acetone added. The effluent was thoroughly dispersed using a small homogeniser (Ultra Turrax), the mixture centrifuged for 10 min at 2000 g to pellet the solids and the supernatant transferred to a 100 ml volumetric flask. The pellet was re-suspended in 20 ml acetone and the process repeated 3 more times. The acetone extract was made up to 100 ml, thoroughly mixed and a filtered (No.1 paper. Whatman) sub-sample (20 ml) stored at –20 °C. A sub-sample (1 ml) of the filtered acetone extract was dried under N_2, made up in HPLC mobile phase, diluted if needed, and assayed by HPLC as above.

Statistics

One tailed Student's paired t-test was used to determine whether carotenoid absorption (within subject) was significantly different ($p < 0.10$, because of the low value of n) between the whole leaf and finely chopped leaf spinach test meals. A one-tailed test was used because it was believed that absorption from the chopped spinach would be greater than from whole leaf spinach because of the more extensive tissue disruption. A 'within subject' correlation of the absorption of β-carotene from whole leaf and chopped leaf spinach was undertaken to test if the magnitude of absorption was volunteer consistent, i. e. whether a higher absorption from whole leaf spinach predicted a higher absorption from chopped leaf spinach, and *vice versa*. Likewise, a correlation for lutein absorption was also undertaken. Fasting plasma concentrations of β-carotene and lutein were correlated with percentage absorption to test if there was a relationship between habitual plasma concentration and absorption from the test meal. Regression analysis was used to assess if physical performance of the meal in the GI tract (lag phase, rate, $t_{1/2}$) was related to absorption.

Data treatment

To characterise the appearance of the spinach meal in the ileal effluent, lutein was used as a marker. The percentage of the original spinach meal collected at each time point was calculated from the total recovered lutein and the amount of lutein found in each collection. Normalised cumulative collection (percentage) of the spinach meals were plotted against time to provide a transit profile of the spinach and the data fitted using a logistic model to calculate the t1/2 of the gastrointestinal residence time. The portion of the curve that appeared to be steeply increasing with time was fitted with a regression model. The slope is equivalent to the effluent production rate, the intercept (x at y = 0 %) the lag time and time for complete passage of the meal (x at y = 100 %).

The total loss of lutein and β-carotene to the effluent and the amount in the spinach meal were calculated and expressed as percentage absorption of the carotenoid content of the meal.

Digesta transit model

The normalised cumulative effluent loss (y) vs. Time (t) data was fitted using the following 2-parameter equation:

$$y = 100 \cdot \left[\frac{e^{-a+b\cdot t}}{1+e^{-a+b\cdot t}}\right]$$

The time taken to reach 50 % loss is given by:

$$t_{1/2} = a/b$$

The slope and y-intercept were found from the linear regression of the data points in the central (linear) section of the normalised cumulative effluent loss vs. time plot. The regression model can be summarised as:

$$y = mt + c$$

where (m) is the slope and is equivalent to the effluent production rate and (c) is the y-intercept. The x-intercept of this line is the lag time and is calculated from:

$$\text{lag time} = -c/m$$

100% transit time (y = 100) can be calculated from:

$$t = \frac{100 - c}{m}$$

TRL area under the curve model.

The areas under the curve (AUC_{oral}) of these plots were calculated using the Altman trapezoidal approximation method [26]. The integrated area under the curves for the TRL response in two of the volunteers who gave complete TRL curves within the 12 h blood sampling period were calculated and compared to the theoretical TRL AUC that would have been obtained if the carotenoids had been given as an intravenous bolus. The half life of the carotenoids in the TRL is not known, thus the theoretical TRL AUC from the bolus was calculated at a number of time points between 2 and 11 min to embrace the chylomicron triacylglycerol $t_{1/2}$ of 2–5 min, up to the chylomicron remnant $t_{1/2}$ of 11 min.

After correcting for the background TRL concentration of carotenoid (lutein and β-carotene), a plot of TRL concentration against time was constructed which represents the TRL response to the oral dose. A single compartment model (Fig. 1) can be assumed with only disposal (k_1) from the TRL fraction, because TRL was the only pool to have an input exclusively (theoretical or real) of newly absorbed carotenoid.

By assuming that a single compartment model (Fig. 1) will approximate chylomicron clearance from the plasma, various plasma half-lives ($t_{1/2}$) were simulated using the SAAM II modelling package [27] to investigate the absorption of an oral dose of carotenoid.

For each $t_{1/2}$, the plasma response to an intravenous (IV) dose was simulated using the above model. The plasma volume of each volunteer was estimated [28] and given as an input parameter in the model. The IV dose was adjusted until the simulated area under the curve (AUC_{iv}) was the same as that found experimentally from the oral dose (AUC_{oral}). Under these conditions, the simulated IV dose was the same as the amount of carotenoid actually absorbed from the oral test meal.

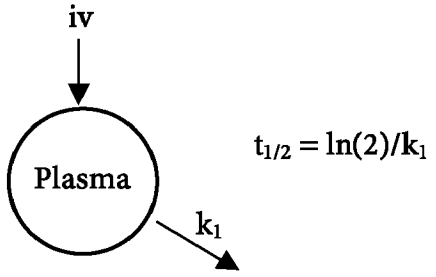


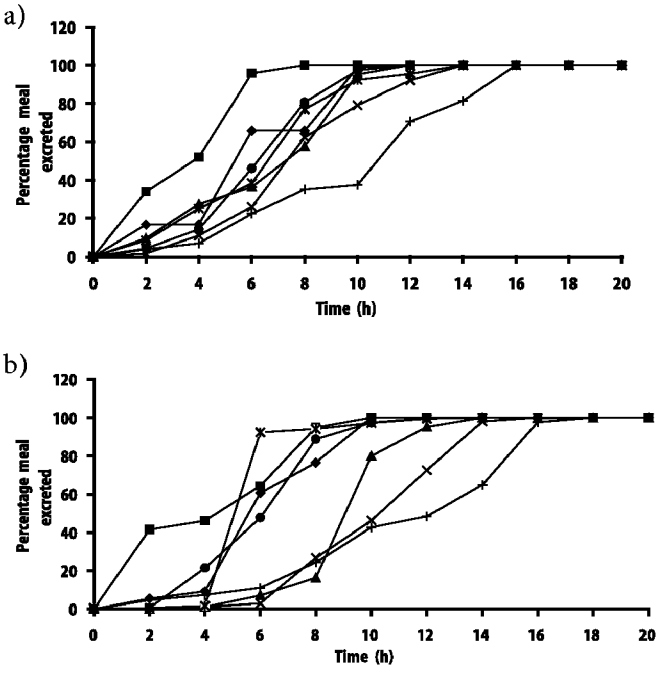
Fig. 1 Single compartment model. k_1 represents the clearance rate of the carotenoids entering the plasma pool as a theoretical intravenous (IV) dose. The half-life ($t_{1/2}$) of the carotenoid in the plasma pool is given by the equation

Results

Meal behaviour in the GI tract

Figs. 2a and 2b show the normalised appearance of spinach meal in the ileal effluent.

The calculated mean lag phase of the initial appearance of the effluent from the chopped leaf spinach meal (2.6 h, range 0.1–4.5 h) was shorter ($p = 0.078$, $n = 7$) than that from the whole leaf spinach meal (3.6 h, range 0–6 h). The mean half-life of the chopped spinach meal in the stomach and small intestinal (SI) tract (6.5 h, range 3.4–10 h) was shorter ($p = 0.08$, $n = 7$) than that for the whole leaf meal (7.4 h, range 4–11.3 h). The mean rate of mass transit through the SI tract as estimated from the slope of the cumulative collection of spinach in the ileal effluent was the same with both meals. The chopped spinach meal passed the whole SI (12.6 h, range 8–16 h) more rapidly ($p = 0.099$, $n = 7$) than the whole leaf spinach meal (13.4 h, range 10–18 h). The transit



Volunteers, A ◆, B ■, C ▲, D ×, E ∗, F ●, G +.

Fig. 2 The normalised appearance of the spinach meals in the ileal effluent of 7 volunteers fed chopped leaf spinach (**a**) or whole leaf spinach (**b**)

time of the whole leaf spinach meal at all time points was about 1 h longer than that of the chopped leaf meal.

Absorption of lutein and β-carotene by mass balance

All the meals contained approximately 15 mg lutein and 10 mg β-carotene, the exact amount being measured by weighed intake and assay of a retained portion of the meal. The mean ratio of lutein: β-carotene was 3:2 in the whole leaf and chopped spinach. Chopping and drip loss from the prepared spinach meals did not affect this ratio.

The percentage absorption of lutein and β-carotene from leaf and chopped spinach is shown in Table 1.

Lutein absorption from whole leaf spinach (mean 44%, range 28–58%) was greater ($p = 0.01$, $n = 7$) than from chopped leaf spinach (mean 26%, range 8–58%) whereas the mean absorption of β-carotene from both meals was similar (chopped 23%, range 4–41%, whole leaf 26%, range 9–58%). Percentage lutein absorption from all meals was weakly correlated ($R^2 = 0.27$, $p = 0.05$, $n = 13$) with lag phase of initial appearance of spinach in ileal effluent by the equation: percentage absorption = 4.6 x lag phase (h) + 20.4. This indicated about 20% lutein absorption with the shortest (>2 h) lag phase and up to 55% absorption with the longest (6 h) lag phase. Residence time in the SI tract altered the ratio of β-carotene: lutein absorbed. There was equal mass absorption of both carotenoids for short lag phase rising to 3:2 lutein:β-carotene at longer residence times. Absorption of β-carotene and lutein were also weakly correlated ($R^2 = 0.43$, $p = 0.01$, $n = 14$); percentage β-carotene absorbed = 0.49 x percentage lutein absorbed + 7.7.

There was no relationship between fasting plasma concentration of lutein or β-carotene and the amount absorbed measured by mass balance.

Table 1 Percentage absorption lutein and β-carotene from spinach (whole leaf and chopped leaf) meals by mass balance in ileostomy volunteers

	Lutein % absorbed		β-Carotene % absorbed	
Volunteer	Whole	Chopped	Whole	Chopped
A	33.6	28.0	22.4	30.0
B	28.0	16.0	23.0	20.6
C	51.0	26.7	32.0	21.0
D	52.7	7.3	8.7	4.3
E	29.6	14.9	13.0	14.7
F	57.0	57.9	52.1	40.9
G	58.5	33.2	33.7	32.5
Mean	44.3*	26.3	26.4	23.4
(SD)	(13.4)	(16.6)	(14.5)	(12.2)

* Whole leaf lutein is significantly greater ($p < 0.05$) than chopped leaf lutein

Plasma and lipoprotein β-carotene and lutein response

Fasting plasma β-carotene (mean ± SD) was 216 ±171 ng/ml and lutein 135 ±72 ng/ml.

None of the volunteers (n = 6) showed a plasma response for β-carotene or lutein with either chopped or whole leaf spinach, probably because the amounts absorbed over the time taken for the spinach to pass through the SI tract were too low (β-carotene, 2.5 mg; lutein 3.75–7.5 mg). There was also no measurable carotenoid response in either LDL or HDL fractions. However, there was a measurable TRL fraction response in 50% of the volunteers (n = 3) for both β-carotene and lutein from chopped and whole leaf spinach, but complete curves were obtained for only 2 volunteers within the 12 h period of blood sampling. The absorption, measured by mass balance in these 2 volunteers, was compared to that predicted at various half life ($t_{1/2}$) values (Table 2).

Table 2 Predicted and measured absorption of carotenoids from spinach meals by TRL response. Lutein and β-carotene TRL absorption kinetics from spinach meals: Predicted percentage absorption from a single compartment model with various values of $t_{1/2}$ and absorption measured by mass balance in two volunteers

	Lutein				β-Carotene			
Volunteer	B	B	G	G	B	B	G	G
Meal Type	WL	CL	WL	CL	WL	CL	WL	CL
$t_{1/2}$ (min)	Predicted Absorption %				Predicted Absorption %			
2	17	68	36	44	18	87	141	58
3	11	45	24	29	12	58	94	38
4	8	34	18	22	9	43	71	29
5	7	27	14	18	7	35	57	23
8	4	17	9	11	5	22	35	14
11	3	12	7	8	3	16	26	10
Measured Absorption%	28	16	58	33	23	21	34	33

WL Whole leaf spinach. *CL* Chopped leaf spinach

In most cases, the measured absorption falls within the range predicted from $t_{1/2}$ values in the range 2–11 min. The exception was the measured absorption of lutein from whole leaf spinach, which was greater than could be accounted for by even the most rapid plasma clearance.

Discussion

Understanding the concept of bioavailability is essential to all involved in food production, nutritional assessment and determination of diet:health relationships. However, the absorption and post-absorptive metabolism of many of the bioactive organic components of foods is complex and not fully understood. The carotenoids provide an excellent example of where too little understanding of the complexity of the behaviour of a food component within the food matrix, during digestion, absorption and clearance and within human tissues, can lead to naïve interpretation of study results.

Faecal mass balance studies are constrained by (a) dietary modification, (b) prolonged sample collection, and (c) the assumption that loss is the same as absorption. Some of these criticisms can be overcome by using modified mass balance methods such as the ileostomy model [20] and TGWM [19].

Interpretation of plasma or plasma fraction carotenoid excursions can only be undertaken with a clear knowledge of the absorption and clearance mechanisms and by sampling the most appropriate 'pool'. Even so, there are inevitably assumptions that need to be invoked and justified when using plasma response models.

Particular care must be taken with chronic dosing when comparing 'relative bioavailability' for four main reasons, (a) the absence of a knowledge of dose response, (b) change in plasma concentration induced by a test food relative to the free compound does not provide an absolute absorption, (c) change or rate of change of plasma carotenoid concentration can be constrained if the doses exceed the capacity of the gut to absorb or the plasma to carry, thus all excessive doses will provoke the same plasma response, (d) at what point during the supplementation period are the relative plasma responses to be measured?

Acute studies also present specific problems. These relate to (a) observing small changes in plasma concentration against a high endogenous background, (b) avoiding the confounding influence of sequestration from and re-export to the plasma pool, (c) lack of knowledge of absorption and clearance kinetics, (d) lack of knowledge of dose response relationship curves.

Newly absorbed carotenoids appear in the plasma chylomicron fraction (TRL), which is virtually free of carotenoids in the fasting state; thus measurement of the TRL response avoids both the problems of quantifying small changes against a high endogenous background and the problems arising from carotenoid trading between other body pools.

The present study sought to quantify β-carotene and lutein absorption from a representative green vegetable, using two experimental systems (together with metabolic modelling) to allow comparison, and more rigorous examination and interpretation, of the data sets obtained.

Mass balance: effects of food structure

Most nutrients have specific absorption mechanisms but many minor lipophilic components are passively absorbed from the gut as an integral part of lipid absorption. Such components, if present in foods of plant origin, must be extracted from their native environment and dissolved in appropriate lipid carriers. Intuitively, breaking up the cellular structure of the food, the presence of lipid, bile salts, lipases and the correct pH should increase the probability of achieving maximum absorption. Absorption from cooked processed foods may be very different by comparison with that from raw; however, disruption of plant cell architecture, beyond that occurring during the relatively mild processing used here, did not influence the proportion of β-carotene absorbed from spinach *in vivo* (Table 1).

The increased absorption of lutein from the whole leaf spinach was unexpected, since it was assumed that the larger particle size would slow down mass transfer to absorbable lipid structures in the stomach and ileum. However, recent studies of carotenoid mass transfer in an *in vitro* gastric and duodenal environment (using samples taken from spinach as fed to human volunteers) demonstrate that whilst the rate and limit of mass transfer are the major controlling factors of transfer to the lipid phase, time is much more crucial for the transfer of lutein [polar] than β-carotene (apolar) (Fillery-Travis. A. Personal Communication). This would explain the enhanced absorption of lutein from the whole leaf spinach and the lack of self-consistency of lutein absorption (Table 1), which might be confounded by unquantified transit rate fluctuations or changes in luminal conditions which affect lutein, but not β-carotene, absorption.

Mass balance: inter- and intra-individual response

Inter-individual β-carotene absorption response to the same food was highly variable but intra-individual response was consistent ($R = 0.887$, $n = 7$, $p = 0.01$) between different forms of the same food (Table 1). A self-consistency of plasma response has also been seen in

chronic supplementation studies [6]. This could be due to two mechanisms: those individuals who exhibit low plasma concentrations of β-carotene (a) absorb less β-carotene, or (b) the β-carotene is absorbed to the same extent but is not retained in the plasma pool. The converse would be true for those showing high plasma β-carotene concentrations.

The data show wide variation between individuals with regard to lutein absorption but, unlike β-carotene, there was no self-consistency between the different forms of spinach meal despite there being a weak correlation between lag phase and amount absorbed ($R^2 = 0.27$, $p = 0.05$, $n = 13$), with the longer residence time doubling the absorption.

Modelling of TRL response

Those individuals that showed a TRL response to β-carotene and lutein, from both chopped and whole leaf spinach, were the same in both cases but were not the individuals that gave the greatest absorption. The fact that there is a measurable TRL response at all levels of absorption (depending on the individual) is a reflection of clearance from the plasma rather than absorption (confirmed by mass balance). Although all the volunteers had 'normal' plasma lipids, turnover was not measured. It would be expected that individuals with rapid clearance of chylomicrons would show the smallest change in TRL concentration of carotenoids because they would not accumulate in the plasma to any measurable extent. Those volunteers with slower lipid turnover may be those that exhibit measurable TRL carotenoid excursions. This might be the reason why the volunteers fell into consistent groups of TRL 'responders' and 'non-responders' despite the fact that carotenoid absorption occurred in all cases.

Two individuals gave complete TRL AUCs for both lutein and β-carotene, over the 12 h of blood sampling, for both whole and chopped spinach meals (Table 2). Using the kinetic model described in this paper, the measured absorption of lutein from the whole leaf meal exceeded that which is predicted from the model, whereas for the chopped leaf meal the measured absorption falls within the range of acceptable $t_{1/2}$ (2–11 min). This indicates that GI transit rate of whole leaf spinach is positively associated with loss of lutein in the GI tract, which contributes to an increased loss (elevated measured absorption) but which is not seen in the TRL response. Alternatively, TRL is not an appropriate measure for newly absorbed lutein, which, because of its more polar nature, may be partially transported in portal blood by a mechanism un-associated with the chylomicrons and thus it was not detected. For the TRL lutein response to chopped spinach the percentage absorption can be predicted if the TRL $t_{1/2}$ is in the range 2–11 min. The range of $t_{1/2}$ does not allow any decision as to whether the lutein is cleared from the TRL in the extrahepatic capillary bed along with the triacylglycerols ($t_{1/2} \approx 2$–5 min), or remains with the chylomicron remnants (mean $t_{1/2} \approx$ 11.5 min).

For β-carotene the measured absorption exceeds that which would be predicted from a TRL $t_{1/2}$ of 11 min (Table 2). This is not surprising because some of the absorbed β-carotene will be converted to retinol in the enterocytes and it will therefore not appear in the plasma as β-carotene. However, if conversion is low (≈10 %) it will not significantly reduce the TRL β-carotene concentration and could still indicate that some of the β-carotene is absorbed in the extrahepatic capillary bed and the remainder being cleared by the liver along with the chylomicron remnants indicating a shorter $t_{1/2}$. Carotenoid losses, here interpreted as absorption because it is assumed that there are no microbial or oxidative losses, may in fact have occurred. If this is the case then absorption by mass balance in the ileostomy model (and oral-faecal model) is an overestimate and $t_{1/2}$ would be longer than that indicated.

Conclusion

Whole leaf spinach has approximately a 1 h longer GI transit time than chopped leaf spinach and this doubles the loss of lutein (25 % → 40 %) but has no effect on β-carotene (25 %). The attenuated delivery of the small amounts of both lutein and β-carotene from the spinach meals did not cause a measurable plasma AUC or responses in LDL and HDL fractions. TRL carotenoid responses were seen in 50 % of the volunteers but in only 2 cases were the whole curves within the 12 h blood sampling window. Future studies with foods should be run for at least 16 h to ensure the complete curves needed for modelling. In both volunteers, the measured absorption of lutein and β-carotene exceeds that predicted from a theoretical TRL $t_{1/2}$ of 11.5 min and this could be a result of luminal losses that are taken as absorption in the mass balance model but which do not appear in the TRL fraction. In the specific case of β-carotene some of the 'loss' will be accounted for by conversion to retinol.

Acknowledgements The authors gratefully acknowledge the financial support of the European Commission Shared Cost Contract CT97–3100 and the UK Ministry of Agriculture, Fisheries and Food. The authors also thank the volunteers who took part.

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EXHIBIT G

TITLE: NATURAL ANTIOXIDANTS AND ANTICARCINOGENS IN NUTRITION, HEALTH, AND DISEASE / EDITED BY J.T. KUMPULAINEN, J.T. SALONEN.
PUBLISHER/PLACE: Royal Society of Chemistry Cambridge, UK :
VOLUME/ISSUE/PAGES: 1999;():296-298 296-298
DATE: 1999
AUTHOR OF ARTICLE: Hovarti
TITLE OF ARTICLE: EXAMINATION OF FLAVONOID.....
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EXAMINATION OF FLAVONOID CONTENT IN HUNGARIAN VEGETABLES

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National Institute of Food Hygiene and Nutrition, Budapest, Hungary.

1 INTRODUCTION

Recently several studies have proved that flavonoids have effect on the human health and thus they are important component of the human diet. The consumption of several grams of flavonoids is recommended.

The aim of the study was a qualitative and quantitative analysis of concentrations of the flavonoids in vegetables produced and consumed frequently in Hungary.

2 MATERIALS AND METHODS

31 selected vegetables were purchased from the local markets in Budapest at a period of their most frequent consumption. The edible parts of the vegetables were used to the examination. After buying the vegetables were immediately cleaned and freeze dried and stored at -18 °C until the analyses.

The flavonols (quercetin, kaempferol, myricetin) and the flanones (apigenin,luteolin) were measured according to Hertog et al.[1] Briefly, flavonoid glycosides were extracted and hydrolyzed to their aglycons with 2.0 M HCl in boiling 50 % aqueous methanol.[1] The resulting aglycons were quatified by RP-HPLC (Perkin Elmer) on a Premisphere C_{18} column (150 x 3.9 mm, 5 µm, Phenomenex, USA) using methanol/phosphate buffer (45/55 v/v, pH 2.4), as a mobile phase and UV detection (370 nm).

3 RESULTS AND DISCUSSION

Table 1 reports the flavonoid content of the fresh vegetables (mg/kg). Quercetin and kaempferol are proved to be the most widespread flavonoids in vegetables. Our present results are similar to Hertog and co-workers' observations but there are some minor differences in relation of the quality and quantity of the compounds, as well.[2] The highest quercetin concentration could be detected in the different types of onion (67.1-171.3 mg/kg) and in spinach (272.2 mg/kg). Hertog and co-workers did not measure any quercetin in spinach.[2] Significant amount of kaempferol was observed in parsnip, leek, new onion, and broccoli (66.4, 45.8, 34.3, and 30.8 mg/kg, respectively).

Table 1
Flavonoid content of vegetables (m

Sample	Quercetin
Purple radish	nd
Black radish	nd
Horse radish	5.7
Red beet	6.7
Onion (old)	121.5
Red onion	171.3
Onion (new)	67.1
Leek	5.0
Cauliflower	1.5
Broccoli	15.4
Kohlrabi	4.0
Brussels sprout	nd
Lettuce	16.3
Crisped lettuce	35.0
Ice lettuce	13.5
Kale	nd
Chinese cabbage	nd
White cabbage	1.6
Red cabbage	9.2
Cucumber	2.4
Tomato	2.7
Sweet pepper	9.4
Californian pepper	5.1
Carrot	3.5
Parsnip	9.9
Swedish turnip	3.2
Celery root	1.8
Parsley leaves	nd
Celery leaves	nd
Dill	74.5
Spinach	272.2

nd: not detectable.

IN HUNGARIAN VEGETABLES

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kers did not measure any quercetin in
served in parsnip, leek, new onion, and
ly).

Table 1
Flavonoid content of vegetables (mg/kg).

Sample	Quercetin	Kaempferol	Myricetin	Luteolin	Apigenin
Purple radish	nd	10.5	nd	nd	nd
Black radish	nd	21.1	nd	nd	nd
Horse radish	5.7	25.7	nd	9.0	nd
Red beet	6.7	nd	nd	18.3	nd
Onion (old)	121.5	2.6	nd	nd	nd
Red onion	171.3	24.3	nd	nd	nd
Onion (new)	67.1	34.5	nd	nd	nd
Leek	5.0	45.8	nd	nd	nd
Cauliflower	1.5	12.5	nd	4.0	nd
Broccoli	15.4	30.8	nd	nd	nd
Kohlrabi	4.0	24.3	nd	13.0	nd
Brussels sprout	nd	12.8	nd	6.7	nd
Lettuce	16.3	nd	10.2	nd	nd
Crisped lettuce	35.0	8.4	nd	3.9	nd
Ice lettuce	13.5	nd	nd	nd	nd
Kale	nd	4.8	nd	nd	nd
Chinese cabbage	nd	7.3	nd	nd	nd
White cabbage	1.6	11.9	nd	4.2	nd
Red cabbage	9.2	nd	nd	6.3	nd
Cucumber	2.4	3.3	nd	nd	nd
Tomato	2.7	8.4	nd	nd	nd
Sweet pepper	9.4	nd	nd	10.7	nd
Californian pepper	5.1	nd	nd	11.3	nd
Carrot	3.5	nd	nd	nd	nd
Parsnip	9.9	66.4	nd	nd	nd
Swedish turnip	3.2	22.7	85.4	nd	154.0
Celery root	1.8	nd	nd	nd	24.1
Parsley leaves	nd	nd	80.8	nd	nd
Celery leaves	nd	nd	43.4	111.4	248.0
Dill	74.5	nd	7.0	nd	nd
Spinach	272.2	nd	nd	66.4	nd

nd: not detectable.

Only five of the vegetables examined in this study contained myricetin, namely swedish turnip (85.4 mg/kg), parsley leaves (80.8 mg/kg), celery leaves (43.4 mg/kg), lettuce (10.2 mg/kg), and dill (7.0 mg/kg). Regrettably the leaves of parsley, celery and dill are consumed as condiments in special Hungarian dishes therefore the participation of these vegetables in the flavonoid intake of the population is probably negligible. Based on the Hertog's observation only fresh broad been can be considered as a natural source of myricetin, but its concentration is low (26 mg/kg).

Significant amount of luteolin could be detected in celery leaves (111.4 mg/kg), spinach (66.4 mg/kg), red beet (18.3 mg/kg), kohlrabi (13.0 mg/kg), and different types of pepper (10.7-11.3 mg/kg). Only three vegetables contained apigenin, namely celery leaves (248.0 mg/kg), Swedish turnip (154.0 mg/kg), and celery root (24.1 mg/kg). Hertog et al. reported luteolin only in red bell pepper (11 mg/kg), and apigenin in celery (108 mg/kg). In a Danish survey[3] apigenin (740 mg/kg) and luteolin (200 mg/kg) were found in celery leaves, and in parsley (apigenin: 1850 mg/kg).

Opposite of our observations, Hertog and co-workers could not detect any of the flavonoids in spinach, red beet, cucumber, carrot and some of brassicas like sauerkraut, white cabbage, swedish turnip and green cabbage.[2] These discrepancies may be due to different cultivars, varietal and seasonal differences.

3 CONCLUSION

The large group of plant polyphenols attracts major interest because of their potential anticarcinogenic and other beneficial properties, presumably based on their function as natural antioxidants. Otherwise the flavonoids are compounds of particular interest because of their high prevalence in foodstuffs. Our investigation proves the presence of significant amount of different flavonoids in selected vegetables frequently consumed in Hungary. These invstigations are a part of our study on a national database of flavonoids in foddstuffs and help to discover the main sources of flavonoids and the estimation of average daily intake of quercetin, myricetin, luteolin, apigenin, and kaempferol in different groups of Hungarian population.

Acknowledgement

The research was supported by the Ministry of Welfare (ETT 052/1996 02), Hungary.

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AVENANTHRAMIDE ANTIOX

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Sweden.

1 INTRODUCTION

Oats grains are rich in lipids ar
dominate (ca 35 % each) which is
content (ca 18 %) improves the s
vulnerable to oxidation. However,
which presumably protects their o
from deterioration, but which also
oat grains as a source of antioxid
ground oat flour was marketed for
food products that are sensitive to

The best known antioxida
and tocotrienols. Measurement of
that most of the tocopherols are lo
in the endosperm.[3] Investigations
antioxidants other than tocopherol
kingdom, but also Δ^5-avenasterol,
oil at 180 °C[4], and a wide ra
antioxidants have been identified
long-chain fatty acids or alcohols[5]
and anthranilic acids.[6-9] The latter
(derived from *Avena*, the latin nam

2 AVENANTHRAMIDES

2.1. Oats

The four most common cinnamic a
acid in combination with fo
hydroxyanthranilic acid, 5-hydroxy
give 16 combinations of avenant
oats. [6-9] It is possible that further
Oat grain contains 500-800 mg/kg
of the individual compounds va
hydroxyanthranilic acid with feru

EXHIBIT H

[JP,2003-164261,A]

Japanese (PDF)

File Wrapper Information

FULL CONTENTS CLAIM + DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS EXAMPLE

[Translation done.]

FULL CONTENTS

[Claim(s)]
[Claim 1]It homogenizes, after grinding foodstuffs which are the targets of extraction and/or juice and making a low-temperature dispersion medium below 60 ** distribute, How to manufacture extract and/or squeezed juice which are characterized by removing extraction slag and/or juice slag if needed after [a basin system of a foodstuffs useful component] extracting and/or emulsifying.
[Claim 2]A method according to claim 1 characterized by a thing for which foodstuffs which are the targets of extraction and/or juice are chosen from coffee, green tea, tea, oolong tea, Pu-Er tea, iron Kannon tea, herb tea, wild

[Translation done.]

grass tea, Chinese medicine tea, cocoa, a vanilla bean, fruits, and vegetables, and which is one at least.
[Claim 3]Foodstuffs used as this object are what extracts a dry matter and serves as luxury goods, A manufacturing method of the extract according to claim 1 or 2 characterized by a thing which is chosen from coffee, green tea, tea, oolong tea, Pu-Er tea, iron Kannon tea, herb tea, vegetable tea, Chinese medicine tea, cocoa, and a vanilla bean, and which is one at least.
[Claim 4]A method given in any 1 paragraph of Claims 1-3 to which a low-temperature dispersion medium is preferably characterized by being a -5-50 ** low-temperature dispersion medium still more preferably at 50 ** or less at less than 60 **.
[Claim 5]a dispersion medium -- water; -- milk; -- dairy-products; -- inside of these -- at least -- one -- a saccharide. A method given in any 1 paragraph of Claims 1-4 characterized by a thing which is chosen from liquid; of sugar-alcohol, a mineral, vitamin, a stabilizer, an emulsifier, and a bacteriostatic in which one was made to distribute and/or dissolve at least, and which is one at least.
[Claim 6]Homogenization continuously target foodstuffs dispersion liquid in a homogeneous valve which has a narrow gap High pressure, A method given in any 1 paragraph of Claims 1-5 being what uses a homogeneous machine provided with a pump made to dip at high speed, shears and/or grinds foodstuffs by a physical impact, and performs extraction and/or emulsification to a basin system of a foodstuffs useful component.
[Claim 7]A method given in any 1 paragraph of Claims 1-5 being that to which homogenization shears and/or grinds foodstuffs and performs extraction and/or emulsification to a basin system of a foodstuffs useful component by a physical impact by the rotation tooth using a homogeneous machine provided with a rotation tooth rotated at high speed.
[Claim 8]Extract with high extraction efficiency and/or juice efficiency in which one degradation is prevented thru/or controlled at least and/or squeezed juice of flavor, quality, and a color tone which are manufactured by a method of a description in any 1 paragraph of Claims 1-7.

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to manufacture of extract and/or squeezed juice.
More particularly, it is related with the method of manufacturing efficiently the extract and/or squeezed juice which controlled oxidization as much as possible for a short time, by using a homogeneous machine.

[0002]
[Description of the Prior Art]When obtaining extract, for example, real coffee extract, industrially conventionally, the process which carries out the hot water extract of the coffee roast beans pulverization thing of the specified quantity with a direct vent type extraction column, a kneader, a decanter, etc. is an example of a usual state. However, the thing for which extraction operation must be repeated for every batch in this process, Even about 6 to 7 weight % is a maximum, and the soluble solid content content of the coffee extract obtained needs to perform a certain concentration operation, for example, vacuum concentration, freeze concentration, spray drying, etc. for obtaining the coffee essence which has a soluble solid content content beyond it.
[0003]Coffee roast beans deteriorate and dissipate quickly the aroma aroma component which it originally has easily by short-time neglect for about 15 to 30 minutes, and the process of the forced cooling of the coffee extract by a refrigerant of the coffee extract which did in this way and was obtained with hot water is also indispensable. It is usually that such hot water extract operation is performed also manufacture of green tea, tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, etc.
A situation does not differ from the case of coffee greatly.

[0004]Thus, in the hot water extract currently performed industrially conventionally, neither astringency nor added flavor called a harsh taste ingredient is avoided, And, [such added flavor] [appear / while raising extraction efficiency / strongly] . The low-temperature extraction method was thought out, acted as Mizuide, and called it coffee (Dutch coffee and common name). [water drip methods extracted while the waterdrop which is the present extraction method is dropped, although coffee with little loss of an aroma aroma component with little astringency exists in a market]

In order to take 3 to 8 hours, and a long time to obtain the extract of 1% or more of soluble solid content, the product development which turns a profit industrially is in a very difficult situation, and is hardly put in practical use.
[0005]On the other hand, if it sees about extraction of green tea as an example of representation of kind tea, the extraction temperature of green tea will usually be about 60-70 **, and, in the case of industrial production, will usually be processed by batch cycles, such as a kneader and a decanter. Especially green tea is known for the influence which it has on the flavor of an extraction condition also in kind tea being great. If in the case of green tea the ingredients eluted in warm water differ and extraction temperature is generally low set up with extraction temperature, If the flavor ingredient which made the subject amino acid, such as L-theanine and glutamic acid, and various aroma components set up extraction temperature highly again, in addition to them, the astringency ingredient which made polyphenol etc. the subject will come to be eluted. Although an astringency ingredient is an important element which forms the flavor of green tea, if eluted superfluously, it will become the flavor which is not preferred.
[0006]Extraction with hot water brings about prompt fading of pigments, such as a green tea chlorophyll, and yellowing, and is known also for causing the fall of color flavor. Although addition of vitamin C is validated at this prevention from fading, that effect is restrictive and addition of superfluous vitamin C brings about the result which is not preferred according to generating of a vitamin smell.
[0007]Therefore, extraction temperature, extraction time, a bath ratio (volume of the extracted water to the amount of use tea leaves comparatively), etc. are adjusted to extraction of green tea, and a condition setup is carried out so that the most desirable flavor may be obtained. However, as long as hot water extracts green tea, fading of an extracted solution of green tea advances, and it does not escape that a desirable aroma quickly peculiar to green tea dissipates. If the usual low-temperature extraction is chosen and extraction temperature is conversely set to this low, since soluble solid content becomes difficult to be eluted, extraction will take a long time to it and it will reduce productivity remarkably. Therefore, the usual low-temperature extraction method

cannot be used for industrial production.
[0008]In the above, although conventional technology has been explained mainly from quality or an organic-functions side, in manufacture of extract, economical efficiency and the field of industrialization are also still more important, and a coffee roast beans pulverization thing is explained about an example about this point.
[0009]Real coffee extract is used as a main raw material of commercial coffee drinks, such as a can, PET, and a paper carton.
At the time of coffee drink manufacture, it is used, carrying out required quantity private extraction, and also a concentration coffee essence is manufactured by vacuum concentration and freeze concentration by making this into a starting material, further, spray drying of this part is carried out with a dryer, and instant coffee is manufactured each time.
Since the quality of this real coffee extract influences a final product greatly, each beverage manufacturer company elaborates the processing condition of coffee roast beans, and is attaining differentiation of goods. Here, the processing condition of coffee roast beans refers to conditions, such as roast, pulverization, and extraction.
Extraction efficiency is mentioned as one index on the extraction condition of coffee roast beans. This is a rate of the soluble solid content weight of the coffee roast beans in a callable extracting solvent (generally water, such as ion exchange water, soft water, and well water) to the weight of the coffee roast beans fed into extractors, such as a direct vent type extraction column, a kneader, and a decanter, and expresses the efficiency of extraction operation.
[0010]generally, highly transparent, if extraction efficiency is set up low -- a coffee scent remains well comparatively. While the extract of the good flavor in which there felt aftertaste refreshed is obtained, it must become weak or diluted coffee extract of a feeling of a body, for taking out coffee flavor with a final product strongly, the amount of the coffee extract used must be increased, and there is a fault to which economical cost becomes high. If extraction efficiency is set up highly, on the other hand, [the coffee extract which coffee extract with a feeling of a body was obtained, and was obtained while comparatively few products designs of the amount used became possible] Are

easy to become cloudy, and aftertaste is made it not only to to cause the exterior fault of coffee oil surfacing on the extract surface, but to produce bitter taste and harsh taste, and having a bad influence on flavor is known.

[0011]Thus, the actual condition is that the trial which raises the extraction efficiency of coffee extract cannot but set up a maximum permissible in cost from the bad influence to the flavor to a final product.

Controlling extraction efficiency to fixed management width causes schedule controlling.

Here, the degree of roast of roast coffee beans, the degree of crushed grain, the expiration date and the extraction temperature of extract, extraction time, cooling temperature, etc. are mentioned as a general factor. In industrial production, it is usually that extraction efficiency is set up to about 22 to 30% in consideration of economical efficiency and the flavor of a product.

[0012]Coffee extract is affected by the influence of oxidization, hydrolysis, heating, etc., and has the character to be easy to deteriorate very in quality. the real coffee in which such a phenomenon carried out drip extraction at home, for example -- a pot etc. -- warming -- when it holds, it is the scent of pure coffee dissipating, setting daily acidity becoming strong etc., and experienced. Therefore, various methods have been examined industrially that the factor which has such a bad influence should be eliminated.

[0013]For example, although extraction of a coffee roast beans pulverization thing is usually performed at about 95 **, the method of providing the coffee drink which has the original flavor of coffee by carrying out at low temperature 90 ** or less as mentioned already is known. To be sure, according to this method, it is possible to reduce the influence of oxidization, heating, etc., and ** is possible for obtaining coffee extract with good flavor once. However, extraction at low temperature causes decline in extraction efficiency, and the yield decreases. Although it is possible to raise extraction efficiency by lengthening extraction time, the fault that the working rate of an extractor falls is not avoided, but the usual low-temperature extraction method is unsuitable to industrialization after all.

[0014]Since this point is improved, it is also possible to raise extraction efficiency by grinding coffee roast beans finely and increasing contact surface area with an extracting

solvent, but. Having a bad influence on flavor and appearance, such as generating of bitter taste and harsh taste, on the other hand at nebula of the coffee extract mentioned above, surfacing of the coffee oil on the surface of extract, and aftertaste is known. Phenomena, such as clogging in the filter mesh in the extractor of coffee roast beans fine particles, may also be generated, and equipment special to removal of these fine particles may be needed.
[0015]It is not only very difficult to secure the extraction efficiency which can bear industrial cost in extraction with the hot water in 70 ** or less although it is the above-mentioned low-temperature extraction, but it only extracts coffee roast beans at the temperature of 70-90 **, A coffee pure scent dissipates promptly, causing the flavor deterioration of **, such as receiving the liquefaction by oxygen in the air of coffee oil, is not avoided, and it has not resulted in essential problem solving.
[0016]Neither a high temperature extraction method nor a low-temperature extraction method may not be satisfied, and it is the same about other foodstuffs as were described above, and neither a high temperature extraction method nor a low-temperature extraction method may not be satisfied in coffee and it mentioned already also in green tea.
[0017]
[Problem to be solved by the invention]As described above, in a high temperature extraction method, degradation of the flavor quality of a product is not avoided but to this, [a low-temperature extraction method] In [extraction efficiency and juice efficiency are bad, and it is not industrial, and] this conventional method, The foodstuffs which are generally the targets of extraction and/or juice, for example, coffee, From single articles, such as green tea, tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, cocoa, a vanilla bean, fruits, and vegetables, or such combination articles, promptly after grinding soluble solid content with a low-temperature solvent, Efficient and continuous, and in order [when using the solvent in which it is very difficult with which extraction and/or to carry out juice for a short time, and they contain protein and lipids, such as milk,] to raise extraction efficiency, high temperature extraction -- not choosing -- it could not but obtain but it had to be dared as the compensation to take the risk of flavor deterioration, such as solidification by heating of protein, and

deterioration of a lipid.
[0018]Thus, in a conventional method, both a high temperature extraction method and a low-temperature extraction method in view of there being a decisive problem, [this invention] It is made in order to newly develop the epoch-making method that the extraction efficiency and juice efficiency which it not only can extract a desirable wind flavor, but can carry out juice in a short time which especially industrialization was also considered are high.
[0019]
[Means for solving problem]This invention is made to achieve the above objects, and, [this invention persons] [by adding a dispersion medium of low temperature instead of high temperature using a homogeneous machine as a result of examination from every direction, and adopting new composition of processing with a homogeneous machine, for the first time] Extract, such as coffee which have neither harsh taste nor astringency and other added flavor, and was excellent in a wind flavor, is obtained extremely for a short time, And if it is in that extraction efficiency -- soluble solid content can collect with high yield -- is very high in that case, and a case of real coffee, In spite of having extracted extract with water, it assumed opalescence which mixed milk, and with extract by the usual extraction method, becoming new foodstuffs which differ in appearance clearly also discovered for the first time.
[0020]It not only can manufacture extract, but by processing with a homogeneous machine using a low-temperature dispersion medium, from non-dried foods, such as fruits and vegetables, it can manufacture squeezed juice, i.e., juice, and this invention is one of the features that extraction and a point in which both sides of juice are possible are also big. And also in juice, quality squeezed juice in which degradation of a wind flavor was controlled like a case of the above-mentioned extraction can be manufactured at a very high yield and juice efficiency.
[0021]And a kind of dispersion medium can also be replaced by dispersion media other than water, for example, milk and others, again, In that case, a thing for which a product which was rich in various kinds of varieties according to a solvent which carries out milk coffee and other use can be efficiently manufactured by very easy

operation, And many new useful knowledge that an unknown product could also be conventionally manufactured depending on a solvent and processing object foodstuffs to be used was acquired for the first time.
[0022]This invention is completed at last based on these useful new knowledge as a result of research. Hereafter, this invention is explained in full detail.
[0023]In order to carry out this invention, after grinding especially pulverizing object foodstuffs, it usually homogenizes. Homogenization continuously target foodstuffs dispersion liquid using the pump made to dip at high pressure and a high speed in the homogeneous valve which has a narrow gap with this physical impact, Foodstuffs are sheared and ground and foodstuffs are processed like the above by the physical impact by the rotation tooth using the thing which performs the extraction and/or the emulsification to the basin system of a foodstuffs useful component, or the rotation tooth rotated again at high speed.
[0024]If homogenization is a device which can carry out the above-mentioned homogenizing step besides being a high-pressure type homogeneous machine, a homogeneous machine of the type to which the high velocity revolution of the rotation tooth is carried out, etc., Shache pump, my RUDA, colloid mill, and others various kinds of commercial items in which a various device is usable are usable suitably.
[0025]In homogenization, it is [more than 20 kg/cm^2] desirable to apply the homogenization pressure more than 100 kg/cm^2 preferably. When homogenization pressure is too low, extraction efficiency falls and it becomes impossible to fully acquire the effect of this invention. Although it does not limit in particular, homogenization is performed above pressure 150 kg/cm^2, and it usually homogenizes in many cases above 500 kg/cm^2.
[0026]In this invention, although processing object foodstuffs are homogenized, foodstuffs need to homogenize, after making a low-temperature dispersion medium distribute in that case. Thus, extraction and/or juice are carried out moreover extremely for a short time efficiently, controlling oxidization controlling generation of added flavor and holding the quality of a useful component by homogenizing under low-temperature conditions.
[0027]Thus, extraction and/or after carrying out juice, in

accordance with a conventional method, separation removal of extraction slag and/or the juice slag is carried out (a liquid cyclone, a clarifier, centrifugal separation, filtration, precision filtration, decantation, etc.), and the extract and/or squeezed juice (juice) which are made into the purpose are obtained. When asking for use for textiles or pulp, for example in the case of vegetable juice or fruits juice, it is not necessary to separate slag completely and the separation of slag itself cannot be performed depending on the case.
[0028]In this invention, it homogenizes under low-temperature conditions using a low-temperature dispersion medium, and less than 60 ** of low-temperature dispersion media [55 ** or less of] 50 ** or less are used still more preferably preferably. Although what is necessary is just the temperature which a solvent does not freeze and it is based also on the kind of solvent about a low-temperature minimum, it is not less than -5 **, and it is usually preferred to consider it as not less than -3 **. Although a 5-20 ** low temperature region is illustrated in the embodiment, specifically, this invention is feasible also in a 2-30 ** low temperature region or the above-mentioned low temperature region. A cooling device may be provided in a homogeneous machine with necessity.
[0029]As a dispersion medium, the dispersion medium of each of these ingredients illustrated to one next at least besides water, milk, and dairy products (fresh milk, skimmilk, whey, sour milk, carrying out reduction whole powdered milk reduction skimmilk powder etc.) which adds one at least is usable.
[0030]a saccharide (glucose, fructose, a shook sirloin, a lactose, and a maltose.) oligosaccharide (a trehalose, a raffinose, lactulose, and a melibiose.) RAKUTO oligosaccharide, galactosaccharide, a fructo oligosaccharide, and other galacto-oligosaccharide and; isomerized sugar; liquid sugar; sugar-alcohol (erythritol.) xylitol, maltitol, and other sorbitol and; minerals (calcium.) Magnesium, sodium, another potassium and; vitamin (vitamin A, B, C, D, E in addition to this); stabilizer (pectin, carboxymethyl cellulose, others); bacteriostatics, such as sucrose fatty acid ester and polyglyceryl fatty acid ester, an emulsifier, a pH adjuster, perfume, a pigment, others.
[0031]Although the extract and/or squeezed juice by which degradation of flavor quality was prevented by

homogenizing various foodstuffs in this invention using a low-temperature dispersion medium using the above-mentioned method are obtained, More than one sort or it of : coffee, the green tea, the tea, Chinese tea (oolong tea, Pu-Er tea, iron Kannon tea, etc.) and herb tea in which the following are illustrated as processing object foodstuffs, wild grass tea, Chinese medicine tea, cocoa, a vanilla bean, fruits, and vegetables

[0032]It is as follows when the embodiment of the manufacturing method of the extract by the low-temperature homogenization concerning this invention and/or squeezed juice is illustrated.

[0033]The foodstuffs which are generally the targets of extraction and/or juice, for example, coffee (Mode 1), Green tea, tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, cocoa, a vanilla bean, Fine-grinding processing of single articles, such as fruits and vegetables, or such combination articles is carried out, -Give homogenization aiming at the extraction and/or the emulsification to the basin system of a foodstuffs useful component after making suitable dispersion media, such as 3 to 50 ** water, distribute, A manufacturing method which obtains the flavor whose extraction and juice efficiency are very high and, which is fresh and the extract which has a color tone removing extraction slag and juice slag by a certain means, and/or squeezed juice.

[0034]The palatability food and drinks generally obtained from extraction of a dry matter, for example, coffee (Mode 2), Single articles, such as green tea, tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, and cocoa, Or after carrying out fine-grinding processing of such combination articles and making suitable dispersion media, such as -3 to 50 ** water, distribute, A manufacturing method which obtains the flavor whose extraction efficiency is very high and, which is fresh and the extract which has a color tone which give homogenization aiming at the extraction and/or the emulsification to the basin system of a foodstuffs useful component, and are characterized by removing extraction slag by a certain means.

[0035](Mode 3) After carrying out fine-grinding processing of the roasted coffee beans and making suitable dispersion media, such as -3 to 50 ** water, distribute, A manufacturing method which obtains flavor whose

extraction efficiency is very high and, which is fresh and extract which has a color tone which give homogenization aiming at extraction and/or emulsification to a basin system of a foodstuffs useful component, and are characterized by removing extraction slag by a certain means.

[0036](Mode 4) Fine-grinding processing of the kind tea, such as green tea, tea, oolong tea, herb tea, wild grass tea, and Chinese medicine tea, is carried out, -Give homogenization aiming at extraction and/or emulsification to a basin system of a foodstuffs useful component after making suitable dispersion media, such as 3 to 50 ** water, distribute, A manufacturing method which obtains flavor whose extraction efficiency is very high and, which is fresh and extract which has a color tone removing extraction slag by a certain means.

[0037](Mode 5) After carrying out fine-grinding processing of the greenstuff, such as a carrot and a tomato, and making suitable dispersion media, such as -3 to 50 ** water, distribute, A manufacturing method which obtains flavor whose juice efficiency is very high and, which is fresh and squeezed juice which has a color tone which give homogenization aiming at extraction and/or emulsification to a basin system of a foodstuffs useful component, and are characterized by removing juice slag by a certain means.

[0038](Mode 6) In a homogeneous valve which generally has a narrow gap, continuously, at high pressure and a high speed, a homogeneous machine is target foodstuffs dispersion liquid a pump made to dip, and with this physical impact, A manufacturing method which obtains extract indicated in any 1 paragraph of the modes 1-5 being the homogeneous machines which shear and grind foodstuffs and can attain extraction and/or emulsification to a basin system of a foodstuffs useful component, and/or squeezed juice.

[0039](Mode 7) A homogeneous machine is provided with a rotation tooth generally rotated at high speed, and with a physical impact by the rotation tooth, Extraction indicated in any 1 paragraph of the modes 1-5 shearing and grinding foodstuffs and attaining extraction and/or emulsification to a basin system of a foodstuffs useful component, and/or a manufacturing method which obtains squeezed juice.

[0040]Manufacture of extract of coffee, i.e., a coffee roast beans pulverization thing, is taken for an example, and this

invention is explained below concretely. That is, pulverization processing of the coffee roast beans is carried out, homogenization aiming at extraction and/or emulsification to a dispersion medium of a foodstuffs useful component is given after distributing this to suitable dispersion media, such as -3 **-50 ** water, coffee extraction fine-particles slag is removed by a certain means, and a coffee extraction method is manufactured.

[0041]According to this method, it becomes possible to raise extraction efficiency which is usually 22 to 27% to about 40%. Usually, since about 30% is made into a limit, the soluble solid content which can be extracted from coffee roast beans can collect about 1.3-time soluble solid content as compared with these. Obtained real coffee extract is assuming opalescence which mixed milk, differs in appearance from extract by the usual extraction method clearly, and can be called new foodstuffs.

[0042]Leakage of this takes place in efficient and the instant of soluble components from the coffee roast beans fine-particles surface under high pressure first with a homogeneous machine, Subsequently, as a result of forming the colloidal particle of an oleophilic ingredient (ingredient mainly named coffee oil generically) among soluble components (emulsification), it is for being tinged with opalescence by the light scattering of a colloidal particle. This nebula does not disappear by centrifugal processing and heat-treatment, either, and holds the very stable suspension. Bitter taste peculiar to hot water extract coffee and harsh taste were not sensed at all, but the freshly ground scent peculiar to coffee has revealed strongly the flavor of the real coffee extract obtained by this method, and under seal conditions, even if it neglects it at ordinary temperature for 24 hours, outstanding its flavor and scent are still maintained.

[0043]As the real coffee extracted with usual hot water was mentioned above on the other hand, extraction efficiency causes surfacing to the coffee extract surface of coffee oil depending on it being not only bad but conditions, and in response to the fact that [oxidization by oxygen in the air] promptly, it causes flavor deterioration. A coffee aroma aroma component usually dissipates promptly in neglect for about 15 to 30 minutes.

[0044]The desired end cannot be gained, even if it

homogenizes when hot water is used so that clearly [this point may also be the same as when it homogenizes and] also from the embodiment which carries out a postscript. That is, although it is the method of mixing extraction feed with hot water, carrying out solid liquid separation after homogenization, and manufacturing extract, in the case where it is coffee, for example, the coffee oil contained in roast coffee beans about 15% emulsifies by homogenization, and shifts to extract. Since the aroma component of the lipophilicity used as the coffee feature scent has melted into coffee oil, the extract containing many coffee oil has coffee flavor desirable originally. However, the extract which coffee oil oxidized very easily and was prepared by the above-mentioned method on the other hand is difficult for obtaining the desirable coffee flavor which oxidization of coffee oil is promoted and is originally expected by being exposed to high temperature by a hot water extract. Existence of the coffee oil contained rather so much depending on conditions may cause flavor deterioration. Thus, this invention is a process with utility value very high as an extraction method of real coffee.

[0045]Coffee roast beans are ground by granulator, in order to usually acquire moderate extraction efficiency. In this stage, a roast coffee-beans pulverization thing is a several millimeters bit, even if this is distributed to a dispersion medium according to this invention and it tries to perform homogeneous processing by high pressure, a bit of coffee roast beans is stuck for a homogeneous valve, and uniformity is not easy. Therefore, in order to perform efficient extraction processing, it is necessary to carry out fine-grinding processing preparatorily to a particle size which can process coffee roast beans with a homogeneous machine. Preliminary grinding of coffee roast beans brings about a result in which preparing to 100 micrometers or less is [particle diameter of 1000 micrometers or less] desirably good. By a device with various preliminary grinding, although it is possible, a stone mill, a hammer mill, a ball mill, a jet mill, a nano mizer, a frost-shattering machine, etc. are mentioned, for example. When flavor of real coffee extract is taken into consideration, it is desirable to use a device with as much as possible little generation of heat at the time of grinding.

[0046]Although uniformity is as stated above, in

pressurizing a homogeneous valve, when a high-pressure type homogeneous machine is used, it is [more than 20 kg/ cm^2] desirable to apply homogenization pressure more than 100 kg/cm^2 preferably. When homogenization pressure is too low, it becomes difficult to exceed 35% and it becomes impossible for an effect of this invention to fully expect extraction efficiency of real coffee extract.

[0047]Although it must wait for details about a mechanism from which the quality characteristic of real coffee extract changes with homogenization a lot to future research, with an operation of powerful shearing of a homogeneous machine, a cavitation, etc., It is made by momentary and efficient leakage of soluble components, and Among these, caffeine, The dissolution to a dispersion medium of hydrophilic components, such as organic acids, such as a chlorogenic acid and quinic acid, and a mineral, Phenomena, such as suspension of dietary fibers, such as colloid emulsification of oleophilic ingredients, such as coffee oil, and a hemicellulose, and discharge of an aroma aroma component which has not received thermal denaturation, happen, and it is thought by conventional hot water extract real coffee extract that flavor, a color tone, and texture which are not seen are appeared.

[0048]In this invention, low-temperature homogenization is carried out, temperature at the time of extraction is one of the important requirements, and less than 60 ** is 55 ** or less preferably like previous statement. Although it does not limit, generally, a suitable temperature region is -3-50 **, and is 10-40 ** still more preferably. When extraction of roast coffee beans is mentioned as an example, if extraction temperature is too low, the dispersibility of roast coffee beans which carried out fine-grinding processing may worsen, and it may interfere with manufacture -- uniform dispersion liquid are not obtained. Decline in extraction efficiency is not avoided, either. If extraction temperature exceeds 50 **, when it will become especially not less than 60 **, flavor deterioration accompanying oxidization of coffee oil stops conversely, making a meaning of this invention remarkably.

[0049]A dispersion medium usable at this invention is not limited to water, but its solvent by which general use is carried out is usable as an extracting solvent for various kinds of food materials like previous statement. If the milk

warmed, for example tends to extract coffee roast beans from use of milk being also very effective, for example, an organic acid leaking it by extraction of coffee roast beans, If the pH of extract is shifted to the acidity side and it comes to be less than pH 6.2, milk protein carries out acid condensation by warming, and normal extraction is difficult for it. Therefore, although addition of pH adjusters, such as sodium bicarbonate, etc. is needed, change [flavor / of an original cafe au lait] is not escaped. However, in this invention, since milk can be extracted with low temperature, if temperature is low, since it is not generated, such acid condensation can reproduce the flavor of the full-scale cafe au lait which blended milk and real coffee at home as it is. The obtained cafe au lait often held the coffee freshly ground scent, and had the coffee-flavored milk flavor of mild aftertaste.

[0050]Since fine extraction slag is contained in the real coffee extract processed with the high-pressure type homogeneous machine, it is necessary to carry out separation removal by a certain method. Although this invention does not prescribe the method, use of a filter, microfiltration, a liquid cyclone, a clarifier, a decanter, etc. is possible like previous statement as a general separation removal method.

[0051]In this invention, improvement in extraction efficiency and the manifestation of a new and characteristic quality characteristic are accepted also in palatability food and drinks generally obtained from extraction of a dry matter, such as tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, and cocoa.

[0052]In the case of green tea and a herb, like coffee, very high extraction efficiency is acquired and extract assumes the beautiful green which is not obtained with warm water. Flavor does not almost have astringency and added flavor, it is the feature to have mild aftertaste and the quality characteristic which reversed old common sense is shown.

[0053]In Chinese medicine tea, it is possible to extract compared with a general hot water extract, without giving a thermal damage to an effective crude drug ingredient. The recovery of an active substance also rises sharply.

[0054]In the case of fruits and vegetables, although squeezed juice is obtained through a juice process, it becomes possible [the juice by this method] also for

carrying out pulverization processing of a dried fruit or the dehydrated vegetables, and pulverizing by frost shattering or other means. For example, in the case of a carrot, the efficiency of juice can increase and the recovery of carotene which is a nutrient in which a carrot is still more important can be raised. Since the obtained squeezed juice has the high carotene content, it assumes skillful orange and flavor is also excellent.

[0055]Thus, this invention enables it to raise sharply extraction of single articles, such as coffee, green tea, tea, oolong tea, herb tea, wild grass tea, Chinese medicine tea, cocoa, a vanilla bean, fruits, and vegetables, or such combination articles, and/or the efficiency of juice. The extract and squeezed juice which were obtained have a quality characteristic with very high respectively utility value.

[0056]

[Working example]Although an embodiment is given to below and this invention is explained to it, thereby, this invention is not limited.

[0057]

[Work example 1]The coffee beans from Colombia roasted to the L value 21 were pulverized so that it might become after pulverization by granulator and might be set to 100 micrometers or less with a mortar. Then, to one copy of pulverized coffee beans, 20 copies of 20 ** desalted water was added, and it uniformed by 150 kg/cm^2 with the high-pressure type homogeneous machine (made by the Sanwa machinery company). The obtained extract performed centrifugal processing for 750 G or 10 minutes. The weight of the supernatant liquid was measured and the soluble solid content was measured with the saccharimeter. And extraction efficiency was calculated from this weight and the value of soluble solid content. Same operation was performed even if it used 20 copies of desalted water (40 **, 60 **, and 90 **). As a contrast article, 20 copies of 20 ** desalted water was added to one copy of pulverized coffee beans, and the sample which performed centrifugal processing for 750 G or 10 minutes was prepared after maintenance for 10 minutes. The sample prepared with 90 ** desalted water was also prepared. Five special panels performed flavor comparison by the sample prepared to 1.0% of soluble solid content.

[0058]Thus, the prepared sample is summarized to below and shown.
(Contrast)
b. 20 ** desalted water extraction RO : 90 ** desalted water extraction (this invention)
**: 20 ** desalted water uniformity extraction NI : 40 ** desalted water uniformity extraction (contrast)
**: 60 ** desalted water uniformity extraction HE : 90 ** desalted water uniformity extraction [0059]A test result is shown in the following table 1. The measurement item is as follows.
A: Extraction efficiency (%)
B: Color tone C : flavor feature (at the time of 1% of coffee soluble solid content preparation)
C1: -- a scent -- C2:bitter taste C3:acidity C4:added flavor C5: -- an overall evaluation [0060]organic-functions evaluation performed five-step evaluation on an absolute scale (1: -- weak/-- bad -5: -- strong/-- good) by five special panels, and used average value as the score.
[0061]
(Table 1)
----------. A B C1C2 C3 C4 C5.
----------. ** 25 Dark brown 3.2 2.0 1.6 2.0 3.4 RO 29 Dark brown 2.8 3.6 2.8 3.4 3.4.
----------. ** 37 Brown 4.0 which became cloudy 2.0 1.4 1.4 4.4 NI 38 Brown 3.6 which became cloudy 2.6 1.8 1.8 4.2.
----------. ** 40 Brown 2.4 which became cloudy 3.0 2.5 2.0 3.2 HE 42 Brown 2.2 which became cloudy 3.4 2.6 2.8 3.0----------
[0062]The extract obtained by homogenization showed the high extraction efficiency exceeding 35%, and the color tone presented nebula by emulsification of coffee oil so that clearly from the above-mentioned result. However, about these flavors, the temperature at the time of extraction influenced greatly, and, as for what was processed at 60 ** and 90 **, the degradation smell was accepted by oxidization of coffee oil. On the other hand, what was processed at 20 ** and 40 ** had a desirable scent, and had the good flavor which is not obtained in the general extraction method. Thus, it was shown that extraction temperature needs to be less than 60 ** for obtaining good flavor by uniformity extraction of coffee.

[0063]
[Work example 2]Green tea was processed with the frost-shattering machine, and fine particles of 10 micrometers or less were obtained. Then, to one copy of pulverized green tea, 20 copies of 15 ** desalted water was added, and it processed by the colloid mill (made by PUC) of the continuous processing type. The obtained extract performed centrifugal processing for 1000 G or 10 minutes. The weight of the supernatant liquid was measured and the soluble solid content was measured with the saccharimeter. And extraction efficiency was calculated from this weight and the value of soluble solid content. Same operation was performed even if it used 20 copies of 95 ** desalted water. As a contrast article, 20 copies of 15 ** desalted water was added to one copy of pulverized green tea, and the sample which performed centrifugal processing for 1000 G or 10 minutes was prepared after maintenance for 10 minutes. The sample prepared with 95 ** desalted water was also prepared. Five special panels performed flavor comparison by the sample prepared to 0.3% of soluble solid content.
[0064]Thus, the prepared sample is summarized to below and shown.
(Contrast)
(**) :15 ** desalted water extraction (**) -- : -- 95 ** desalted water extraction (this invention)
(**) :15 ** desalted water uniformity extraction (contrast)
(**) : 95 ** desalted water uniformity extraction [0065]A test result is shown in the following table 2. The measurement item is as follows.
a: Extraction efficiency (%)
b: Color tone c : flavor feature (at the time of 1% of soluble solid content preparation)
c1: -- a scent -- c2:bitter taste c3:astringency c4:taste c5: -- an overall evaluation [0066]organic-functions evaluation performed five-step evaluation on an absolute scale (1: -- weak/-- bad -5: -- strong/-- good) by a marks method by five special panels, and used average value as the score.
[0067]
(Table 2)
----------. a b c1c2 c3 c4 c5.
----------. (b) 22 -- dark green 3.6 3.0 2.2 2.4 2.4 -- dark green 3.2 [somber (**) 28] 4.24.8 2.0 1.6. ----------. (**) 38 Skillful dark

green 4.0 1.6 1.4 3.6 4.0. Dark green 3.2 [somber ---------- ---------- (**) 38] 4.0 4.6 3.0 2.0----------[0068]as compared with the contrast article whose both are 38% and whose extraction efficiency of the sample extracted by uniformity at 15 ** and 95 ** is a general extraction method, the difference clear-came out so that clearly from the above-mentioned result. However, the thing with strong bitterness and astringent taste in which the sample which carried out uniformity extraction at 95 ** has a bad color tone was the feature, and the overall evaluation was low. On the other hand, the sample which performed uniformity extraction at 15 ** presented skillful green and very good flavor, and it had a quality characteristic which is not acquired in the old extraction method.

[0069]

[Work example 3]The carrot which performed blanching treatment was processed with the frost-shattering machine, and fine particles of 500 micrometers or less were obtained. Then, 25 ** desalted water was mixed with these fine particles at a rate of 1:1, and it uniformed by 200 kg/cm^2 using the high-pressure type homogeneous machine (made by the Sanwa machinery company). The obtained liquid performed centrifugal processing for 1000 G or 10 minutes, and removed a part for pulp. The efficiency of juice was computed from the weight and the soluble solid content content of the obtained carrot juice. That is, the amount of soluble solid content obtained from 100g of carrots was calculated. Squeezed juice was condensed to 42% of soluble solid content by the evaporator, and performed quality evaluation. The sample prepared by the same method as centrifugal processing or subsequent ones was used as a contrast article based on the squeezed juice obtained with the meat chopper. Five special panels performed flavor evaluation. The beta carotene content contained in concentration squeezed juice was analyzed by the HPLC method.

[0070]The obtained result is shown in the following table 3. However, the measurement item is as follows.

(a): Juice efficiency (%)

(b): Color tone (c) : flavor feature (c1) : it is fragrant (c2) and is :sweet taste (c3):overall evaluation (d):beta carotene (mg/100g).

[0071]organic-functions evaluation performed five-step evaluation on an absolute scale (1: -- weak/-- bad -5: -- strong/-- good) by a marks method by five special panels, and used average value as the score.
[0072]
(Table 3)
----------. (a) (b) (c1) (c2) (c3) (d). ----------. Pair ** 5.2 orange 2.2 3.0 3.2 28. ---------- ---------- this invention 8.2 -- skillful orange 4.0 4.6 4.4 48----------
[0073]The sample obtained by the juice method by this invention had high juice efficiency as compared with the contrast article, and has collected many beta carotene so that clearly from the above-mentioned result. It was checked that sweet taste and a scent are excellent also in [it is strong and] flavor.
[0074]
[Effect of the Invention]Think out that this invention homogenizes object foodstuffs under low-temperature conditions for the first time, and this invention Extraction of foodstuffs, and/. Or hot water is not used [in which high concentration extraction and/or juice are possible at low-temperature solvents, such as cold water, in carrying out juice], Production of a process even including mere extraction and not only distribution to the basin system of a foodstuffs useful component but emulsification stabilization of the oleophilic leached moiety is enabled continuously efficient again, And the obtained extract and/or squeezed juice do not spoil at all the flavor and color tone which foodstuffs originally have with heating, but have advantages, such as making it possible to manufacture industrially the food and drinks of the flavor which carried out private extraction at home. This invention is just an industrially very useful and new invention.
[0075]The foodstuffs in which this invention is generally the target of extraction or juice so that clearly also from the above-mentioned embodiment, For example, efficiently in points, such as a color tone, flavor, and an extraction ingredient, [vegetables / coffee, tea, herb tea, Chinese medicine tea, fruits] [soluble solid content] [extraction or the extract which carried out juice and was moreover obtained, and squeezed juice] An old concept is wiped away and it becomes possible to provide attractive quality

for consumers. Therefore, a manufacturer and consumer side can tell both sides the epoch-making production technology which gives a merit. The effect which can also carry out low-temperature homogenization twice or more, and was further excellent in that case is generated.

[Translation done.]

Report Mistranslation | Japanese (whole document in PDF)

DERWENT-ACC-NO: 2003-715545
DERWENT-WEEK: 200419

TITLE: Manufacturing extract and/or squeezed liquid useful as food/beverage, involves grinding raw material, homogenizing, dispersing in medium, extracting, emulsifying and removing dregs and/or squeezed dregs

INVENTOR: IMAZAWA T ; KUBOTA Y ; NAKATSUBO T ; YOSHIMURA Y

PATENT-ASSIGNEE:

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MEIJI MILK PROD CO LTD	MEIP

PRIORITY-DATA: 2001JP-365005 (November 29, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
JP 2003164261 A	June 10, 2003	JA
WO 03045157 A1	June 5, 2003	JA
AU 2002365357 A1	June 10, 2003	EN

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP2003164261A	November 29, 2001	2001JP-365005	
AU2002365357A1	November 21, 2002	2002AU-365357	
WO2003045157A1	November 21, 2002	2002WO-JP12179	Based on

INT-CL-CURRENT:

TYPE	IPC	DATE
CIPS	A23 F 3/16	20060101
CIPS	A23 F 3/18	20060101

CIPS A23 F 3/34 20060101
CIPS A23 F 5/24 20060101
CIPS A23 F 5/26 20060101
CIPS A23 L 1/30 20060101
CIPS A23 L 2/38 20060101

ABSTRACTED-PUB-NO: JP 2003164261 A
BASIC-ABSTRACT:

NOVELTY - A method for manufacturing extract and/or squeezed liquid, involves grinding raw material, homogenizing, dispersing in medium at less than 60degreesC, extracting, emulsifying and removing dregs and/or squeezed dregs.

DESCRIPTION - An INDEPENDENT CLAIM is also included for high efficiency extract and/or squeezed liquid, which are obtained by preventing deterioration of flavor, quality and color tone of the extract.

USE - As food/beverage.

ADVANTAGE - The method efficiently provides extract and/or squeezed liquid in a short period of time.

ABSTRACTED-PUB-NO: JP 2003164261 A
EQUIVALENT-ABSTRACTS:

BIOLOGY

Preferred Materials: The raw materials are selected from coffee, green tea, black tea, oolong tea, herb tea, wild grass tea, chinese medicine tea, cocoa, vanilla, fruits or vegetables. The dispersion medium has low temperature of less than 50degreesC preferably -5-50degreesC. The dispersion medium is water, cow's milk dairy products, liquid of saccharides, sugar alcohol, mineral, vitamin, stabilizer, emulsifier and bacteriostatic. The mixture is homogenized using homogenous machine equipped with pump, which pours dispersion liquid at high voltage and high speed continuously in the homogenous valve. The valve has narrow gap.

A roasted coffee bean (in weight parts) (1) was ground and mixed with desalted water (20) at 20degreesC. The above mixture was homogenized at 150 kg/cm2 and centrifuged for 10 minutes to obtain extract. The obtained extract was found to have favorable flavor and color tone.

TITLE-TERMS: MANUFACTURE EXTRACT SQUEEZE LIQUID USEFUL FOOD BEVERAGE GRIND RAW MATERIAL HOMOGENISE DISPERSE MEDIUM EMULSION REMOVE DREG

DERWENT-CLASS: D13

CPI-CODES: D03-H01;

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: 2003-197143

EXHIBIT I

(19) 대한민국특허청(KR)
(12) 공개특허공보(A)

(51) 。Int. Cl. [7]
A23C 9/12

(11) 공개번호 특2003- 0022942
(43) 공개일자 2003년03월19일

(21) 출원번호 10- 2001- 0055799
(22) 출원일자 2001년09월11일

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심사청구 : 있음

(54) 식물성 액상 발효유(醱酵乳) 및 분말 발효유의 제조방법

요약

본 발명은 콩과 쌀 (백미 또는 현미) 등의 식물성 식품을 주재료로 하여 액상 발효유를 제조하고, 또한 이를 동결건조시켜 분말형의 발효유를 제조하는 방법 및 이렇게 하여 제조된 액상 발효유 및 분말형 발효유에 관한 것이다. 본 발명에 따르면, 식물성 발효유는 비피도박테리움속 (Bifidobacterium)의 균주 1종과 락토바실러스속 (Lactobacillus bulgaricus)의 균주 1종을 두유 (豆乳; 콩을 열처리하여 마쇄하고, 비지를 분리 제거하여 제조)와 미유 (米乳; 백미 또는 현미를 열처리하여 당화시킨 후, 마쇄하고 착즙하여 제조)가 일정하게 혼합된 배지에 접종하고 발효시키는 단계를 포함하는 방법에 의해서 제조된다. 이와 같이 제조된 식물성 발효유는 보존성 및 이동성을 향상시키기 위하여, 발효가 종료된 발효유를 동결 건조시킴으로써 유산균의 생존뿐 아니라 발효유 내의 영양성분의 파괴를 최소화한 새로운 형태의 유산

균 발효식품을 제조한다.

대표도

도 1

색인어
액상 발효유, 분말형 발효유, 두유, 미유, 비피도박테리움, 락토바실러스

명세서

도면의 간단한 설명

도 1은 두유와 미유의 혼합비율에 따른 기호도 (숫자는 응답한 관능평가자의 수)를 나타낸 것이다.

도 2는 발효시간에 따른 비피도박테리움 비피덤 (Bifidobacterium bifidum)과 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus)의 생균수 변화를 나타내는 그래프이다.

도 3은 발효 및 동결건조 종료 후, 보관시간에 따른 생균수의 변화를 나타내는 그래프이다.

발명의 상세한 설명

발명의 목적

발명이 속하는 기술 및 그 분야의 종래기술

본 발명은 콩과 쌀 등의 식물성 식품을 주재료로 하여 비피도박테리움속 (Bifidobacterium)의 균주 및 락토바실러스속 (Lactobacillus)의 균주를 사용하여 액상 발효유를 제조하는 방법 및 이와 같이 제조된 액상 발효유를 동결 건조시켜 분말 발효유를 제조하는 방법 및 이러한 방법으로 제조된 액상 또는 분말형 발효유에 관한 것이다. 본 발명에 따르면, 콩과 쌀 등의 식물성 원료를 사용하여 발효유를 제조하기 때문에 동물성 우유에서 야기될 수 있는 문제점이 없이 영양적으로 유익한 발효유를 제공할 수 있으며, 또한 제조된 발효유를 동결 건조하여 분말상으로 제조할 수 있기 때문에 저장성 및 이동성을 높임과 동시에 유용한 유산균의 생존율을 유지시킬 수 있다는 잇점이 제공된다.

최근에, 식물성 식품에 대한 관심이 높아지면서, 우유와 같이 동물성 원료를 사용하는 대신에 콩과 쌀 등을 주재료로 이용한 식물성 음료수의 개발 및 수요가 증가하고 있다. 동물성인 우유의 경우, 인체 내에서 동맥경화 및 고혈압 등과 같은 성인병의 원인이 된다고 알려진 포화지방산 및 콜레스테롤의 함량이 높고, 또한 유당불내증을 가진 사람에게는 음용이 곤란한 경우가 많은 것으로 알려져 있다. 그러나, 식물성 음료, 예를 들어, 두유나 쌀 음료 등에는 인체에 유용한 불포화지방산이 포함되어 있고 콜레스테롤은 거의 포함되어 있지 않다고 알려져 있으며, 유당을 포함하고 있지 않으므로, 유당불내증의 문제없이 누구나 손쉽게 음용할 수 있다는 장점이 있다. 이러한 식물성 식품의 장점을 이용하여 동물성 우유의 대체품으로 두유가 시판되고 있는 실정이다. 또한, 쌀을 주성분으로 한 음료수도 최근 들어 각광을 받고 있다.

한편, 콩을 원료로 하여 만든 두유에서 한 단계 더 나아가, 두유를 비피도박테리움 (Bifidobacterium) 및 락토바실러스 (Lactobacillus) 등의 유산균으로 발효시켜 식물성 발효유를 제조하고 동물성 요구르트의 대체용으로 개발하고자 하는 노력이 있었으며, 그 결과 여러 가지 방법들이 제시되었다. 그러나, 이들은 주로 콩으로부터 두유를 제조하는 방법 또는 두유 특유의 풀 냄새(grassy smelling)를 제거할 목적으로 여러 가지 유산균을 사용하여 불쾌한 콩의 풍미 및 발

효풍미를 매스킹하는 방법들에 관한 것들이다. 그러나, 이들 공지의 방법으로 제조한 발효두유의 경우, 콩 자체의 영양학적 측면을 고려한 것은 없었으며, 또한 제조된 발효유의 보존 및 운반에 있어서의 유산균 사멸에 대해 고려한 것은 아직 없었다. 즉, 콩 등의 두류에는 인체가 필요로 하는 아미노산들이 다량 포함되어 있기는 하나, 필수 아미노산의 하나인 메치오닌이 제1차 제한아미노산으로 알려져 있고, 트립토판 등의 다른 필수아미노산은 충분히 함유되어 있어서, 콩 단백질만을 섭취하는 것은 영양학적으로 바람직하지 못하다. 한편, 쌀 단백질에는 트립토판 등이 부족하기는 하나 콩 단백질에 부족한 메치오닌이 다량 함유되어 있다. 따라서 본 발명자들은 이들 두 가지 식물성 식품을 적당히 혼합하여 아미노산 보충효과를 나타내도록 하면, 영양학적으로 우수한 식물성 음료뿐 아니라, 이를 이용한 유산균 발효유의 제조할 수도 있을 것으로 판단하였다.

또한, 발효유의 경우에는 일반적으로 냉장보관을 하더라도 시간이 경과함에 따라 발효에 관여했던 유산균의 생존율이 현저하게 감소하는 경향을 나타내며, 따라서 당해기술분야에서는 발효유의 이러한 보존상의 문제점을 해결하는 방법을 개발하고자 시도가 계속되었다.

본 발명자들은 상기한 바와 같은 기존의 선행기술에서 밝혀진 문제점들을 해결함으로써 발효유의 보존 시에 시간이 경과하여도 발효에 사용되는 유산균의 생존율이 저하하지 않고, 또한 제조된 발효유의 이동성을 향상시키기 위한 방법을 광범하게 연구하였으며, 그 결과 본 발명에서는 두유와 미유가 적절히 혼합된 배지에 상술한 유산균을 접종하여 발효시키고, 또한 발효 종료 후에 동결 건조시킴으로써, 유산균의 생존율을 최대화하고 이동성 및 보존성을 획기적으로 향상시키고자 하는 목적을 성공적으로 달성할 수 있음을 확인하고 본 발명을 완성하게 되었다.

발명이 이루고자 하는 기술적 과제

본 발명의 목적은 종래의 두유 및 두유 발효유가 가진 영양학적 문제점을 보완하기 위하여, 두유와 미유를 적당량 혼합하여 새로운 식물성 음료를 제조하고, 나아가 이에 비피도박테리움 (Bifidobacterium) 및 락토바실러스 (Lactobacillus) 속의 유산균을 이용하여 발효시킴으로써 영양성 및 기호성이 우수한 새로운 액상 발효유를 제공하는 것이다.

본 발명의 또 다른 목적은 상기한 바와 같이 제조된 식물성 발효유를 동결 건조시켜, 액상 발효유에서보다 유산균의 생존율을 향상시키고, 보존성 및 이동성이 우수한 분말 발효유를 제공하는 것이다.

발명의 구성 및 작용

상기에서 설명한 바와 같이, 본 발명은 콩과 쌀 등의 식물성 식품을 주재료로 하여 액상 발효유를 제조하는 방법 및 이러한 액상 발효유를 동결 건조시켜 분말 발효유를 제조하는 방법에 관한 것이다. 이하에서는 이러한 본 발명의 방법을 구체적으로 설명한다.

1. 두유의 제조

본 발명에서 '두유'는 종래 방법에 의해 통콩 또는 탈지콩으로부터 제조된 것이거나 또는 시판되고 있는 두유를 의미한다. 본 발명에서는 예를 들어, 콩을 약 25- 40℃의 물에서 약 5- 10시간 침수시켜, 콩을 수화시킴과 동시에 가용성 성분인 사포게닌 및 당류의 일부를 제거하고, 이렇게 처리된 콩을 100℃의 물 중에서 1- 2분간 가열처리한 후, 분쇄하여 슬러리화하고, 슬러리로부터 불용성 성분을 분리 제거한 후, 수득한 슬러리를 약 80℃ 이상에서 약 5분간 방치하고, 여과, 원심분리 등의 통상의 방법을 이용하여 비지와 두유를 분리시킴으로써 두유를 제조할 수 있다. 이렇게 분리된 두유는 약 100℃에서 약 10분간 살균 처리하고 냉각시킨 후에 본 발명에 따라 발효에 사용한다.

2. 미유의 제조

한편, 본 발명에 따르는 액상 발효유의 제조 시에 또 다른 원료로 사용되는 미유는 백미 또는 현미 또는 이들의 혼합물을 사용하여 제조한다. 본 발명에서 미유로는 백미 및 현미를 가열 처리한 후 바로 슬러리화 한 것을 미유로 사용할 수도 있고, 또는 슬러리화 하기 전에 당화 단계를 거쳐 당화된 미유를 사용할 수도 있다. 당화효소원으로는 시중에 판매되고 있는 엿기름을 사용할 수도 있고, 발아현미를 사용할 수도 있다. 미유를 당화시켜 사용할 경우의 장점으로는, 일차적으로 백미 또는 현미를 당화시켜 유산균이 발효에 사용할 수 있는 단당류 또는 이당류를 생성함으로써 발효 시에 첨가하는 당류의 양을 줄일 수 있으며, 또한 첨가하는 당류보다 당화시켜 얻은 당류가 유산균이 보다 효과적으로 발효에 사용한다는 점 등이다.

본 발명에서 미유는 예를 들어 다음과 같은 방법에 의해서 제조된다. 즉, 우선 백미 또는 현미에 물을 가하여 가열처리하고, 여기에 물을 첨가하면서 분쇄하여 슬러리화 하고, 수득된 슬러리로부터 착즙 또는 원심분리와 같은 방법으로 불용성 성분을 제거하여 미유를 수득한다. 본 발명에 따르는 발효방법에서는 수득된 미유를 다시 100℃에서 약 10분간 살균 처리한 후, 상기에서 제조한 두유와 일정비율로 혼합하여 사용한다. 또 다른 방법으로, 가열 처리한 백미 또는 현미를 슬러리화하기 전에 엿기름 또는 발아현미 등의 당화 효소로 처리하여 당화시킬 수도 있다. 이를 위해서는 우선 엿기름 또는 발아현미를 25℃ 정도의 물에서 1시간 정도 진탕 배양하여 효소를 추출하고, 원심분리 등으로 불용성 성분을 제거한다. 이렇게 제조된 당화 효소를 백미 또는 현미와 혼합하여 약 60℃에서 약 3시간 진탕 배양하면서 당화시킨다. 당화가 종료되면 여과, 원심분리 등으로 불용성 성분을 제거하고, 앞에서 제조한 두유와 적당한 비율로 혼합하여 발효에 사용한다.

본 발명에 따라 발효를 수행할 때에는 상기 1 및 2에서와 같이 수득한 두유 및 미유를 그대로 사용할 수도 있으나, 유산균이 이용할 수 있는 당류를 일정량 첨가하여 사용함으로써 발효를 촉진시키는 것이 더 바람직하다. 이러한 목적으로는 유산균이 사용할 수 있는 당류라면 어느 것이라도 사용할 수 있으나, 본 발명에서는 바람직하게는 설탕 또는 올리고당, 더욱 바람직하게는 설탕을 사용할 수 있다. 즉, 당화시키거나 당화시키지 않은 경우 모두, 두유와 미유를 혼합시킨 다음에 설탕을 첨가하여 최종당도가 약 18 브릭스 (Brix) 정도가 되게 하여 발효에 사용할 수 있다.

본 발명에서 두유와 미유는 고소한 맛, 신맛 등의 기호도, 색상 및 미감 등을 고려하여 적절한 비율로 혼합시켜 사용할 수 있으나, 일반적으로 두유와 미유를 1:10 내지 10:1의 비로 혼합하여 사용한다. 바람직하게는 두유와 미유는 2- 8: 8- 2의 비로 사용할 수 있으며, 가장 바람직하게는 8:2의 비로 사용한다.

3. 유산균 발효

본 발명에 사용하는 비피도박테리움속 (Bifidobacterium)에 속하는 유산균으로는 이 속(屬)에 속하는 균주라면 어느 것이라도 사용할 수 있으며, 예를 들어 비피도박테리움 비피덤 (Bifidobacterium bifidum), 비피도박테리움 롱굼 (Bifidobacterium longum), 비피도박테리움 브레베 (Bifidobacterium brevae), 비피도박테리움 인판티스 (Bifidobacterium infantis) 등을 들 수 있으며, 가장 바람직하게는 비피도박테리움 비피덤 (Bifidobacterium bifidum)을 사용한다. 한편, 본 발명에서 락토바실러스균 (Lactobacillus)으로는 공지 또는 시판균주들 중의 어떤 것이라도 사용할 수 있다. 이에 속하는 균주의 예로는, 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus), 락토바실러스 아시도필러스 (Lactobacillus acidophilus), 락토바실러스 카제이 (Lactobacillus casei) 등이 있으며, 가장 바람직하게는 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus)를 사용한다.

이들 두 가지 균주는 두유 및 미유의 혼합물에 각각 별도로 접종하여 발효시킨 다음에 혼합시켜 사용하거나, 모두 함께 접종하여 유산균 발효처리를 수행할 수도 있으나, 본 발명에서 바람직하게는 두 가지 균주를 두유와 미유의 혼합물에 함께 접종하여 발효를 수행한다. 본 발명에서, 이들 균주는 각각의 균주에 해당하는 통상의 배양조건 및 방법을 사용하여 순수하게 배양한 후, 접종하여 스타터 (starter)를 만들어 사용하거나, 또는 동결건조 상태로 시판되고 있는 균주를 사용할 수도 있다. 접종량은 상기한 바와 같이 제조한 스타터의 경우에는 발효액을 기준으로하여 1% 정도의 농도로 하며, 동결건조균을 접종할 경우에는 초기 균수가 약 10^5 개/㎖ 이상이 되도록 한다. 이어서 유산균이 접종된 두유와 미유의 혼합물을 37℃에서 약 10시간 이하, 바람직하게는 약 7시간 동안 발효시킨다. 발효처리는 기존의 발효우유제조에 사용하고 있는 것과 같은 발효장치 또는 발효탱크를 그대로 사용할 수 있다. 이때, 유산균발효를 산소가 있는 상태에서 행하게 되면 이취가 생성될 수 있다는 보고가 있으므로, 가능하면 발효용기의 헤드스페이스(headspace)를 최소로 하기 위하여 용기를 가득 채운 상태에서 발효를 하는 것이 유리하다.

제조된 본 발명의 식물성 발효유는 교반하여 냉각시킨 후, 용기에 주입하여 액상 요구르트 같은 음료제품으로 제조할 수 있다. 또한, 본 발명에 의해 제조된 제품에는 외관, 기호도 등에 적합하도록, 필요에 따라 본 기술분야에서 식용으로 통상적으로 사용되는 향료, 감미료, 착색료 및 안정화제 등을 포함한 추가의 성분을 첨가할 수 있다. 예를 들어, 액상 발효유에 과실성분을 첨가하여 과실타입의 제품으로 제조할 수도 있다.

4. 분말형 식물성 발효유의 제조

상기한 바와 같이 제조한, 액상 발효유는 보존성 및 이동성을 보완하기 위하여 동결건조시켜 분말형 발효유로 제조할 수도 있다. 이를 위해서는 우선 - 80℃에서 제조된 액상 발효유를 충분히 동결시킨 후, 동결건조기에서 건조시킨다. 동결건조는 바람직하게는 - 45℃ 이하의 온도에서 압력은 10 토르(torr) 이하로 하여 수행한다. 동결건조가 완료된 발효유는 분쇄하여 분말상태로 만든 후에, 그대로 용기에 충전시키거나, 또는 압축하여 타블렛 (tablet) 형태로 제조할 수도 있다. 이렇게 동결처리된 발효유는 음용시에 다시 물 또는 기존의 음료수에 용해시켜 액상 발효유 상태로 복원하여 음용하거나, 또는 분말상태 그대로 복용할 수도 있다.

이러한 방법으로 제조된 분말형 발효유에는 건조 전의 액상 발효유와 거의 동일한 수의 유산균이 생존하며, 따라서 액상 발효유를 마시는 것과 동일한 효과를 얻을 수 있다.

실시예

본 발명은 이하의 실시예에 의해서 더욱 상세히 설명된다. 이들 각각의 실시예는 본 발명의 명확한 이해를 목적으로 제공된 것으로 본 발명의 취지와 범위를 벗어나지 않는 한, 다양하게 변형시킬 수 있으며, 이들 실시예가 본 발명의 범위를 어떤 식으로든 제한하는 것은 아니다.

실시예 1

(1) 두유 및 미유의 제조

콩 700g을 약 25℃의 물 3ℓ 중에서 약 5시간 동안 침수시킨 후에, 100℃의 물 3ℓ 중에서 1- 2분간 가열처리하였다. 그 후, 믹서로 분쇄하여 슬러리화하고, 슬러리로부터 여과하여 불용성 성분을 분리 제거한 후, 수득한 슬러리를 약 80℃의 온도에서 약 5분간 방치하고, 여과 및 3,000rpm에서 원심분리하여 비지와 두유를 분리시켜 두유 약 2.1ℓ 를 제조하였다. 수득된 두유를 약 100℃에서 약 10분간 가열하여 살균처리하고 실온으로 냉각시켰다.

별도로, 엿기름 100g을 약 25℃의 물 400 mℓ 에서 1시간 정도 진탕 배양한 후, 원심분리하여 불용성 성분을 제거하고 당화효소 약 300mℓ 를 수득하였다. 이렇게 수득된 당화효소 250mℓ 을 백미 100g에 넣고, 약 60℃에서 약 3시간 동안 진탕하면서 가열하고, 여기에 물을 부으면서 분쇄하여 슬러리화하였다. 수득된 슬러리를 여과하고 8,000 rpm에서 원심분리하여 불용성 성분을 제거함으로써 미유 약 350㎖를 수득하였다. 수득된 미유를 다시 100℃에서 약 10분간 가

열하여 살균처리하였다.

(2) 발효

상기 (1)에서 제조된 두유와 미유를 8:2의 비로 혼합한 혼합물 400㎖에, 하룻밤 동안 적당한 고형배지에서 활성화시킨 비피도박테리움 비피덤 (Lactobacillus bifidum) 및 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus) 각각의 스타터를 1% (v/v)의 농도로 첨가하여 37℃에서 약 7시간 동안 발효시켰다. 수득된 발효유를 교반하여 냉각시킨 후, 용기에 주입하여 액상 요구르트를 제조하였다.

(3) 분말형 식물성 발효유의 제조

상기 (2)에서와 같이 제조한, 액상 발효유를 - 80℃에서 약 2시간 동안 동결시킨 후, - 45℃ 이하의 온도 및 10 토르 이하의 압력으로 설정된 동결건조기 내에서 동결건조시켰다. 동결건조된 발효유를 분쇄하여 분말형의 식물성 발효유 약 100g을 제조하였다.

실시예 2

상기 실시예 1의 (1)에서 제조된 두유와 당화시킨 미유를 다양한 비율로 혼합하여 실시예 1의 (2)에 따라 액상 발효유를 제조하고 기호도를 조사하였다.

실시예 1의 (1)에서 제조한 두유 및 미유에 각각 에 당도가 18 브릭스에 도달할 때 까지 설탕을 첨가하여 보정하였다. 이렇게 제조된 두유와 미유를 각각 5:5, 6:4, 7:3, 8:2, 9:1, 10:0의 비율로 혼합하고, 여기에 유산균을 접종하여 발효시켰다. 약 7시간의 발효 후, 제조된 발효유의 기호도를 조사하기 위하여 관능평가를 실시하였다. 그 결과를 도 1에 나타내었다.

도 1에 나타낸 결과로부터, 색을 제외한 모든 항목에서, 두유와 미유를 8:2로 혼합한 것이 신맛, 고소한 맛, 질감 등을 포함한 전반적인 면에서 가장 우수하다고 판단되었다.

실시예 3

실시예 1의 (2)의 방법으로 발효를 개시한 후, 시간의 경과에 따라 유산균의 생균수에 있어서의 변화를 측정하였다. 발효를 개시한 후에 시간별로 샘플을 취하여 희석도말법을 이용하여 비피도박테리움과 락토바실러스 각각의 생균수를 측정하였다. 그 결과를 도 2에 나타내었다.

도 2에 제시된 결과로부터 알 수 있는 바와 같이, 실시예 1의 (2)에서 제조한 발효유는 약 7시간 정도의 발효시간으로 발효가 완료되었으며, 이후 30시간이 경과하더라도 생균수의 변화는 없었다. 따라서 이러한 결과로부터, 위의 발효조건에서 발효는 10시간 이하로 충분하다는 것을 알 수 있었으며, 비피도박테리움과 락토바실러스 모두 거의 동일한 생균수를 나타내는 것을 알 수 있었다.

한편, 탈지분유를 사용하여 동일한 방법과 유산균을 사용하여 발효유를 제조하고 생균수를 측정한 결과 발효완료 후, 약 5 X 10^8 세포/㎖를 나타내었다. 이러한 균수는 위에서 식물성 원료를 사용한 발효유와 거의 유사한 생균수를 보이므로, 본 발명에서 제조한 식물성 발효유도 기존의 우유를 사용한 발효유의 유용 균의 섭취라는 기능을 충분히 대신할 수 있다는 사실을 알 수 있었다.

실시예 4

실시예 1의 (2)에 따라 제조된 액상 발효유를 실시예 1의 (3)의 방법에 따라 동결 건조시켰다. 건조가 완료된 후, 건조시킨 것과 건조시키지 않은 액상의 발효유 각각을 4℃에 보관하면서 유산균의 생존율을 시간별로 조사하였다. 그 결과를 도 3에 나타내었다.

발효를 종료하고 동결 건조시킨 분말상태의 발효유는, 시간이 경과함에 따라 초기균수와 거의 변화가 없는 생균수를 보임을 알 수 있었다. 동일한 실험을 상온에서 실온에서 실시하였을 때, 액상과 분말상 모두 생균수의 변화는 냉장 보관한 것들에 비해 다소 차이가 있었으나, 거다란 차이가 없이, 초기의 생균수를 적어도 일주일 이상은 유지함을 알 수 있었다. 또한 건조한 발효유의 경우 물을 넣어 다시 액상으로 전환하여도 맛이나 풍미에서는 건조전의 액상 발효유와 차이를 찾아낼 수 없었다.

발명의 효과

이상 설명한 바와 같이, 본 발명에서 두유와 미유를 사용하여 제조한 식물성 액상 및 분말상 발효유는, 기존의 우유를 사용한 발효유와 거의 같은 수준의 유산균수를 얻을 수 있어서 동물성 식품을 건강에 유익한 식물성으로 대체할 수 있다. 또한, 이렇게 제조된 식물성 액상 발효유를 동결 건조시켜, 유산균의 생존율이 거의 변하지 않으면서 보존성 및 이동성을 현저하게 향상시킬 수 있다.

(57) 청구의 범위

청구항 1.

두유와 미유의 혼합물을 비피도박테리움속 (Bifidobacterium) 의 균주 및 락토바실러스속 (Lactobacillus) 의 균주와 혼합배양하여 발효시킴을 특징으로하여 액상 발효유를 제조하는 방법.

청구항 2.

제 1 항에 있어서, 두유와 미유를 1:10 내지 10:1의 비로 혼합하여 사용함을 특징으로 하는 방법.

청구항 3.

제 2 항에 있어서, 두유와 미유를 8:2의 비로 혼합하여 사용함을 특징으로하는 방법.

청구항 4.

제 1 항에 있어서, 미유가 백미 또는 현미 또는 이들의 혼합물을 당화시켜 수득한 당화된 미유임을 특징으로 하는 방법.

청구항 5.

제 1 항에 있어서, 균주와 함께 배양하기 전에 두유와 미유에 설탕 또는 올리고당을 첨가하여 최종당도가 18 브릭스가 되도록 조정함을 특징으로 하는 방법.

청구항 6.

제 1 항에 있어서, 비피도박테리움속 (Bifidobacterium) 의 균주가 비피도박테리움 비피덤 (Bifidobacterium bifidum), 비피도박테리움 롱굼 (Bifidobacterium longum), 비피도박테리움 브레베 (Bifidobacterium brevae) 및 비피도박테리움 인판티스 (Bifidobacterium infantis) 로 구성된 그룹으로부터 선택되고, 락토바실러스속 (Lactobacillus) 의 균주는 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus), 락토바실러스 아시도필러스 (Lactobacillus acidophilus), 락토바실러스 카제이 (Lactobacillus casei) 로 구성된 그룹으로부터 선택됨을 특징으로하는 방법.

청구항 7.

제 6 항에 있어서, 비피도박테리움속 (Bifidobacterium) 의 균주로 비피도박테리움 비피덤 (Bifidobacterium bifidum) 이 사용되고, 락토바실러스속 (Lactobacillus) 의 균주로는 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus) 가 사용됨을 특징으로하는 방법.

청구항 8.

제 7 항에 있어서, 비피도박테리움 비피덤 (Bifidobacterium bifidum) 과 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus) 가 각각 스타터 형태로 1%의 농도로 발효에 사용됨을 특징으로하는 방법.

청구항 9.

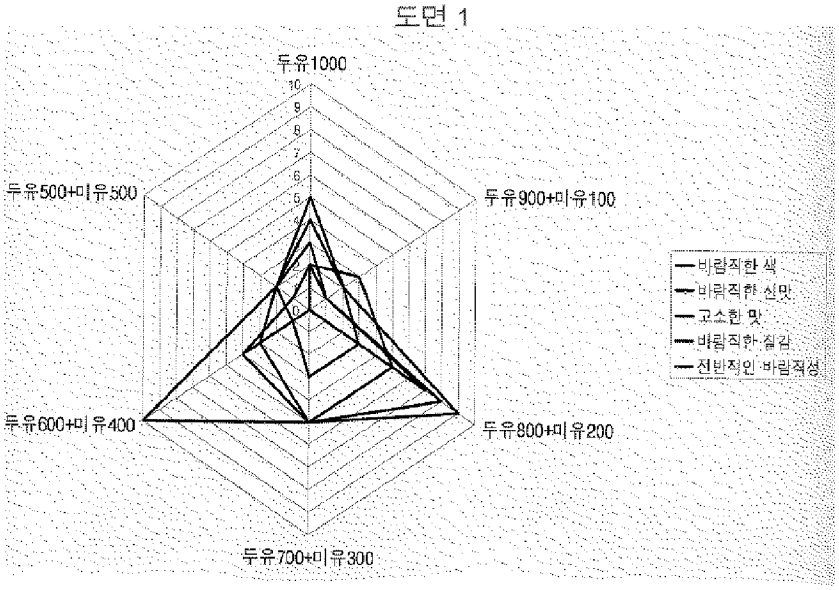
제 7 항에 있어서, 비피도박테리움 비피덤 (Bifidobacterium bifidum) 과 락토바실러스 불가리쿠스 (Lactobacillus bulgaricus) 를 동결건조된 형태로 각각 초기균수 10^{5}/㎖의 비로 발효에 사용함을 특징으로하는 방법.

청구항 10.

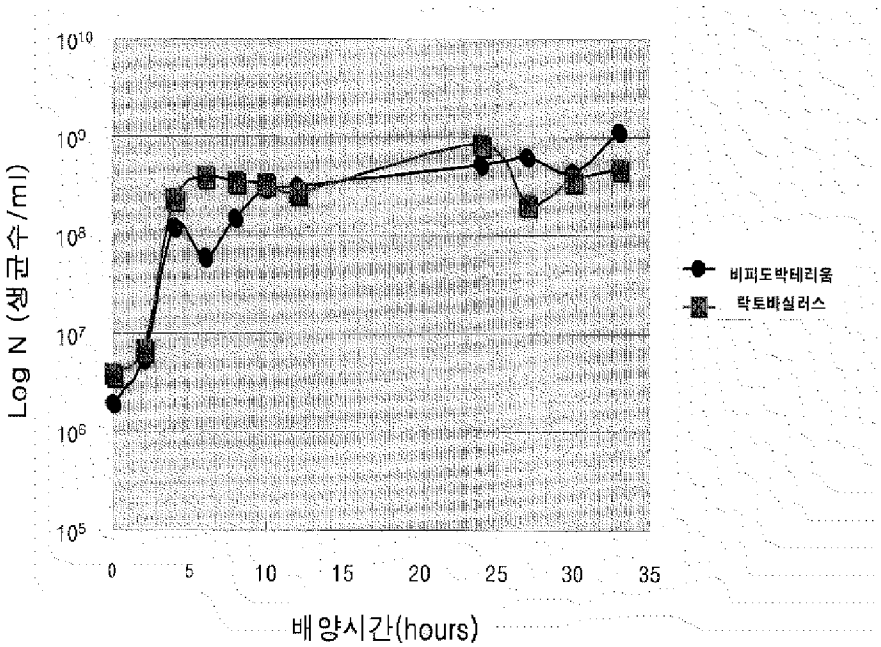
제 1 항에 있어서, 제조된 액상 발효유를 추가로 동결건조시켜 발효유를 분말형으로 제조함을 특징으로 하는 방법.

도면

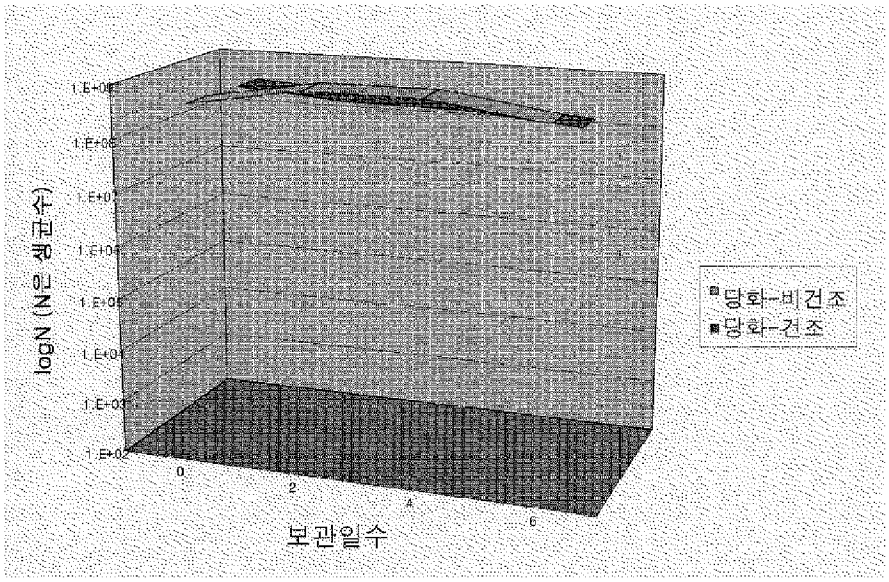
도면 1



도면 2


Log N (생균수/ml)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
5
10
15
20
25
30
35
배양시간(hours)
비피도박테리움
탁토바실러스

도면 3


logN (N은 생균수)
보관일수
당화-비건조
당화-건조

(19)The Korean Intellectual Property Office (KR)
Unexamined Patent Application (A)

(51) Int.Cl. 6
A23C 9/12

Application No	10-2001-0055799
Application Date	2001-09-11
Publication No	KR2003-0022942.
Publication Date	2003-03-19
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Examination	Requested
Title of Invention	A process for preparing the liquefied vegetablefermented milk and powdered fermented milk

Abstract

The invention relates to the main materials the vegetable food including the bean etc. pee (the Cynanchi Radix or the brown rice). And it and the youghurt beverage is manufactured. It is about the method, for this being freeze-dry let moreover and manufacturing the fermented milk of powdery the youghurt beverage and the in this way manufactured powdery fermented milk. According to the invention, vegetable the fermented milk is manufactured with the method for including the step that inoculates the strain one kind of the Bifidobacterium and strain one kind of the Lactobacillus species (Lactobacillus bulgaricus) in soy oil (豆乳: bean is heat-treated and it defaces. It asks, it eliminates and manufactures separation) and the culture medium in which rice milk (after 米乳: Cynanchi Radix or the brown rice is heat-treated and it lets be sugared, it defaces and it juice-squeezes and it manufactures) are regularly mixed and fermented. In this way, in order that vegetable the manufactured fermented milk improves the integrity and portability, the lactic acid bacterial fermentation product of the new form which minimizes the break down of the nutrient component within not only the alive of the lactobacillus but also the fermented milk by letting freeze-dry the fermented milk in which the fermentation is completed, is manufactured.

Representative Drawing(s)

Fig. 1

Keyword(s)

The youghurt beverage, powdery fermented milk, soy oil, rice milk, bipidobacterium, lactobacillus.

Description

※ Brief Explanation of the Drawing(s)

Figure 1 shows the symbol according to the mixing ratio of the soy oil and rice milk is (the number of sense valuer which number answers).

Figure 2 is a graph showing the vialble cell change of the Bifidobacterium bifidum according to the fermentation time and lactobacillus bulgaricus.

Figure 3 is a graph showing the change of the viable cell count according to the keeping time after the fermentation and freeze-drying termination.

※ Details of the Invention

※ Purpose of the Invention

※ The Technical Field to which the Invention belongs and the Prior Art in that Field

The invention relates to the main materials the vegetable food including the bean etc. pee. And it is about the liquid or the powdery fermented milk manufactured with the method, for manufacturing the youghurt beverage by and using the strain of the Bifidobacterium and strain of the Lactobacillus species the method for the in this way manufactured youghurt beverage being freeze-dry let and manufacturing the powdered fermented milk and such method. According to the invention, provided are the advantage can offer the fermented milk it nutritiouses without the problem of being caused by the animal nature milk because of manufacturing the fermented milk by using the vegetable material including the bean etc. pee, profitable, and that it is able to make maintain the survival rate of the at the same time useful lactobacillus with elevation because of the moreover manufactured fermented milk being freeze-dried and manufacturing with the powdered.

Recently, while the concern about the vegetable food is enhanced, the development and demand of the vegetable beverage which the bean etc. pee it uses is used for main materials increase the animal material like the milk. The case in which drink difficults manies and it is known as to the man having the lactose intolerance the saturated fatty acid which is caused by of the adult disease and content of the cholesterol including the arteriosclerosis and hypertension etc. high in case of the animal nature milk in the human body. But the cholesterol the useful the unsaturated fatty acid for vegetable drink a , for example, the human body including the soy oil or the rice beverage etc is included has the advantage that it is known as because of not being nearly included. And the lactose is not included. Therefore, it easily can drink without the problem of the lactose intolerance as anyone. The soy oil goes on sale to the replacement of the animal nature milk by using the advantage of this vegetable food. Moreover, except the beverage the rice to the main component recently receives the foot light.

In the meantime, the effort for the soy oil being fermented with the lactobacillus including the Bipidobacterium (Bifidobacterium) and lactobacillus etc. and to manufacturing vegetable the fermented milk and developing as for substitution of the animal nature yogurt furthermore had in the soy oil in which the bean was to the raw material and made one-stage. And many methods were consequently reindicated. But these the things about the flavor of the method for mainly manufacturing the soy oil from the bean or the bean unpleasant by using various lactobacilluses as the purpose of eliminating the grass smell (grassy smelling) of the soy oil characteristic and the methods masking the fermentation flavor. But in case of the fermented soymilk manufactured with these publicly known methods, it had no to consider the nutritional side of the bean itself. And it yet had no to consider for the lactobacillus extinction in the conservation of the moreover manufactured fermented milk and carrying. That is, in the pulses crops including the bean etc, amino acids that the human body to need are included the large amount. But the one of the essential amino acid methionine is known as as the first limited amino acid. The other essential amino acid including the tryptophan etc. is enough contained. It is unable to desirable to the dietetics to ingest only the soybean protein. In the meantime, in the pee protein, the tryptophan etc. are insufficient but the insufficient methionine is contained in the soybean protein the large amount. Therefore, if it befittingly mixed these two kinds of vegetable foods and these inventors showed the amino acid supplement effect, it decided to could manufacture of the lactobacillus fermentation milk using this not only vegetable the excellent drink as the dietetics.

Moreover, in case of the fermented milk, although it kept in refrigeration, as the time passed, the tendency that the survival rate of the lactobacillus taking part in the fermentation remarkably diminished was shown generally. And in order to develop the method for solving problem on this conservation of the fermented milk in the technical field, attempt was therefore continued.

The method the survival rate of the lactobacillus used for the fermentation does not fall even if the time passes in the conservation of the fermented milk since these inventors solve problems known through the existing prior art as described above, and for improving the portability of the moreover manufactured fermented milk. And extensively, it studied. And the above-described lactobacillus was inoculated in the culture medium in which the soy oil and rice milk were appropriately mixed and it consequently fermented in the invention. It let freeze-dry after moreover, the fermentation end. In that way it confirmed to successfully could accomplish the purpose for to maximizing the survival rate of the lactobacillus and conspicuously improving portability and integrity and the invention was completed.

▩ The Technical Challenges of the Invention

An object of the present invention are to provide the new youghurt beverage with a superior nutritional and taste it manufactures by thus using the lactobacillus in the Bipidobacterium (Bifidobacterium) and lactobacillus the new vegetable drink the soy oil and rice milk are mixed the appropriate amount the conventional soy oil and the dietetics problem that the fermented soybean milk has are supplemented.

It is still another object of the present invention to provide the powdered fermented milk with a superior integrity and portability the survival rate of the lactobacillus is improved than the youghurt beverage vegetable the manufactured fermented milk is freeze-dry let as described above.

▩ Structure & Operation of the Invention

As described above, the invention relates to the main materials the vegetable food including the bean etc. pee. And it is about the method for and manufacturing the youghurt beverage and the method for such youghurt beverage being freeze-dry let and manufacturing the powdered fermented milk. Hereinafter, such method of the present invention is explained specifically.

1. The manufacture of the soy oil.

In the invention, it is manufactured with the conventional method from the whole soybean or the non-fat bean or 'soy oil' implies the soy oil going on sale. In the invention, the bean is swamped for example in the water of about 25-40℃ about 5-10 hours. A part of the saccharides and the fusible element sapogenin is eliminated with hydrating the bean. After the in this way processed bean is heating processed among the water of 100℃ 1-2 discrimination, it shatters to pieces and it makes slurry. After the insoluble component is eliminated from the slurry separation, the obtained slurry is neglected in about 80℃ or greater about for 5 minutes. By disuniting the bean-curd dregs and soy oil by using the normal method including filtering, centrifuge etc. the soy oil can be manufactured. In this way, in the soy oil separating is about 100℃, after sterilizing about for 10 minutes and cooling, it uses according to the invention in the fermentation.

2. The manufacture of the rice milk.

In the meantime, by using the Cynanchi Radix, the brown rice, or their mixture, the rice milk used in the manufacture of the youghurt beverage followed into the invention as and, the other raw material manufactures. In the invention, after processing heating the Cynanchi Radix and brown rice to the rice milk, it can use as the rice milk to immediately make slurry. Before making slurry, the glycosylated rice milk can be used in other words after the glycosylating step. The malt sold to on the market can be used as the saccharogenic amylase. The germinated brown rice can be used. The Cynanchi Radix or the brown rice is be sugared let first and since the lactobacillus is created monosaccharide or the disaccharide can use in the fermentation amount of the saccharides added can be reduced in the fermentation by the advantage of the case of the rice milk being be sugared let and using. And the saccharides which lets be sugared than the saccharides which it moreover adds and obtained is etc. that the lactobacillus more effectively uses in the fermentation.

In the invention, the rice milk is for example manufactured with the method as follows. That is, firstly, the water is added to the Cynanchi Radix or the brown rice and it processes heating. While here adding the water, it shatters to pieces and it makes slurry. The insoluble component is eliminated from the obtained slurry to method such as juice and centrifuge and the rice milk is obtained. In the fermentation method followed into the invention, after the obtained rice milk is again in 100℃ about for 10 minutes sterilizing process, it mixes to the soy oil and the constant rate which in the above case manufactures and it uses. And before making slurry the Cynanchi Radix or the brown rice processed heating to the other method, it processes as the diastatic enzyme including the malt or the germinated brown rice etc. and it can let be sugared. 1 hour extent shaking culture and enzyme is extracted firstly for this from the water of about 25℃. The insoluble component is eliminated to centrifuge etc. In this way, the manufactured diastatic enzyme is mixed with the Cynanchi Radix or the brown rice and while in about 60℃ about 3 hours shaking culture, it lets be sugared. The insoluble component is eliminated to filtering, centrifuge etc. if saccharification is completed. It mixes to the soy oil and the appropriate rate which it in front of manufactures and it uses in the fermentation.

According to the invention, when being proceed the fermentation, the soy oil and the obtained rice milk can be used like that 1 and 2. But it more desirables to expedite the fermentation by the fixed amount addition and using the saccharides which the lactobacillus can use. If the saccharides which the lactobacillus can use, although anything, it can use as this purpose. But the sugar can be used preferably more preferably in the invention the sugar or oligosaccharide. That is, after letting be sugared or mixing everyone, and the soy oil and rice milk in case of not letting be sugared, the sugar is added and for the final sweetness degree, about 18 brix is and it can use in the fermentation.

In the invention, it mixes to the proper rate and the soy oil and rice milk can use in consideration of the taste, filing a suit the sourness, including, preference, the color and sensuousness etc. But the soy oil and rice milk are mixed to the ratio of 1:10 to 10:1 and it generally uses. Preferably, the soy oil and rice milk can use as the ratio of 2-8:8-2. And it more preferably uses as the ratio of 8:2.

3. Lactobacillus fermentation.

If the strain belonging to this inside, although anything, it can use as the lactobacillus belonging to the bifidobacterium used in the present invention. And for example, the Bifidobacterium bifidum, the Bifidobacterium longum, the Bifidobacterium breve (Bifidobacterium brevae), the Bifidobacterium infantis etc can be in come. And the Bifidobacterium bifidum is used more preferably. In the meantime, in the invention, it can use as the Lactobacillus bacteria although it feels among the public notice or marketing strains. Thus, the lactobacillus bulgaricus, the Lactobacillus acidophilus, the Lactobacillus casei etc. for example have of the strain belonging. And the lactobacillus bulgaricus is used more preferably.

After respectively separately inoculating in the mixture of the rice milk and soy oil and fermenting, it mixes and these two kinds of strains use, or it altogether together inoculates and the lactobacillus fermentation processing can be proceed. But two kinds of strain is inoculated together in the mixture of the rice milk and soy oil and the fermentation is proceed preferably in the invention. In the invention, after these strains purely cultivate by and using method the normal culture tub corresponding to each strain, it inoculates and the starter is made and it uses, or the strain going on sale to the lyophilized state can be used in other words. The initial bacteria number is about 10 the freeze-drying bacteria is inoculated the inoculum size as described above as described aboves in case of the starter manufactured based on the fermentation liquid to the concentration of about 1%.5It in order to become over the / ㎖. Subsequently, the mixture of the rice milk and the soy oil in which the lactobacillus is vaccinated is fermented preferably in 37℃ less than about 10 hours for about 7 hours. The fermentation treatment like that can use the fermenting device or the fermentation tank like using in the existing fermentation milk making. At this time, the report that off-flavor can become if the lactobacillus fermentation is done in the state where the oxygen has has. Therefore, it favorables to full ferment container in the filled state in order to the head space (headspace) of the fermentation container to minimum if it possibles.

After the manufactured vegetable the fermented milk of the present invention ***s and it cools, it injects into container and it can manufacture with the beverage manufactures thing same like the liquid yoghurt. Moreover, the additional component including the outer tube in the product manufactured according to the present invention, the perfume, sweetener, and pigment and stabilizer etc can be added. The perfume in order that symbol suitables for etc., is generally used according to need in this technical field as edibility. For example, the component of fruit is added in the youghurt beverage and it can manufacture with the product of the fruit type.

4. The manufacture of the powdery vegetability fermented milk.

As described above, in order to supplement the integrity and portability, it lets freeze-dry and the youghurt beverage manufactured can manufacture with the powdery fermented milk. After the manufactured youghurt beverage is frozen firstly enough for this in -80℃, it desiccates in the freezing dryer. It 10 Torr this harrow and as to freeze-drying, pressure is preferably proceed at a temperature of -45℃ or less. After the fermented milk in which freeze-drying is completed shatters to pieces and it makes with the powdered state, it like that charges in container, or it compresses and it in other words can manufacture in the form of the tablet. In this way, it again dissolves in the water or the existing beverage and it is restored to the former state in drink to the youghurt beverage state and the frozen fermented milk drinks, or it in other words like that can take the powdered state.

In the powdery fermented milk manufactured with this method, the same lactobacillus as the youghurt beverage of the drying former of number survives. And the effect the youghurt beverage is drunk, similar can be obtained therefore.

Working example.

The invention is more particularly explained with the working example of less than. With these each working examples being offered the understanding of being clear of the present invention to purpose the purport of the present invention and range are not escaped. It variously can metamorphose. And these working examples does not restrict the scope of the present invention to any kind of type.

Working example 1.

(1) The manufacture of the rice milk and soy oil.

After the bean 700g was swamped among the water 3ℓ of about 25℃ for about 5 hours, it processed heating among the water 3ℓ of 100℃ 1-2 discrimination. Thereafter, it shattered to pieces to mixer and it made slurry. After it filtered from the slurry and the insoluble component was eliminated separation, the obtained slurry was neglected at a temperature of about 80℃ about for 5 minutes. It separated centrifugal in filtering and 3,000rpm and the bean-curd dregs and soy oil were disunited and the soy oil about 2.1ℓ was manufactured. The obtained soy oil was heated in about 100℃ about for 10 minutes and it sterilized and it cooled to the room temperature.

Separately, in the water 400 m ℓ of about 25℃ the malt 100g, it separated centrifugal after 1 hour extent shaking culture heartburnings and the insoluble component was eliminated and the diastatic enzyme about 300m ℓ was obtained. In this way, the obtained diastatic enzyme 250m ℓ was put into the Cynanchi Radix 100g. It heated while being mixed in about 60℃ for about 3 hours. While here pouring the water, it shattered to pieces and it made slurry. By the obtained slurry being filtered and separating centrifugal in 8,000 rpm and eliminating the insoluble component the rice milk about 350㎖ was obtained. The obtained rice milk was heated again in 100℃ about for 10 minutes and it sterilized.

(2) Fermentation.

The Bifidobacterium bifidum (Lactobacillus bifidum) and the lactobacillus bulgaricus each starter activated for one night in the appropriate solid medium were added in the soy oil manufactured in (1) and the mixture 400 ㎖ mixing the rice milk to the ratio of 8:2 to the concentration of 1% (v/v) and it fermented in 37℃ for about 7 hours. After the obtained fermented milk was ***ed and it cooled, it injected into container and the liquid yoghurt was manufactured.

(3) The manufacture of the powdery vegetability fermented milk.

After the youghurt beverage manufactured (2) was frozen in -80℃ for about 2 hours, it let freeze-dry to pressure less than temperature less than -45℃ and 10 Torr in the fixed freezing dryer. The fermented milk supplied separately was to pieces shattered and vegetable the fermented milk about 100g of powdery was manufactured.

Working example 2.

The rice milk let be sugared was mixed with the soy oil manufactured in (1) of above statement example 1 to the various rate and the youghurt beverage was manufactured according to (2) of the working example 1 and preference was examined.

Until the sweetness degree arrived in to 18 brix, the sugar was added in each and it revised in the soy oil and the rice milk manufactured from (1) of the working example 1. In this way, the soy oil and the manufactured rice milk were mixed to the rate of the respective 5:5, 6:4, 7:3, 8:2, 9:1, 10:0. The lactobacillus was inoculated here and it fermented. The sensory evaluation was enforced in order to examine the preference of the manufactured fermented milk after the fermentation of about 7 hours. The result was shown for fig. 1.

In the overall side including the thing mixing the soy oil and rice milk from the result shown for fig. 1 in all items except for colour to 8:2 is the sourness, the taste filing a suit, and texture etc, it was determined because of most excellenting.

Working example 3.

After the fermentation began to the method of (2) of the working example 1, the change in the viable cell count of the lactobacillus was measured according to the pass of the time. After begining the fermentation, by

withdrawal and using the dilution coating method, Bipidobacterium and lactobacillus each viable cell count were measured at hourly. The result was shown for fig. 2.

As shown in it could know from the result reindicated in fig. 2, in the fermented milk manufactured from (2) of the working example 1, the fermentation was completed to the fermentation time of about 7 time obout. And the change of the viable cell count had no although 30 hours thereafter passed. Therefore, in the fermentation condition from such result, it could know to show the viable cell count which was nearly similar both bipidobacterium the fermentation could know the it was enough 10 hours this harrow thing and lactobacillus.

In the meantime, about 5 X 10 after the method similar by using the skimmed milk powder and fermentation done the viable cell count is measured the fermented milk is manufactured the lactobacillus is used8The cell / ㎖ was shown, this microbial content seened the viable cell count which was nearly similar in the upper part to the fermented milk using the vegetable material. Therefore, the fact that vegetable the fermented milk manufactured from the invention enough could replace function called the intake of the useful bacteria of the fermented milk using the existing milk could be known.

Working example 4.

The youghurt beverage manufactured according to (2) of the working example 1 was freeze-dry let according to the method of (3) of the working example 1. After the aridity was completed, while safekeeping the fermented milk of the liquid which it did not desiccate with the thing desiccated in 4℃, the survival rate of the lactobacillus was examined in hourly. The result was shown for fig. 3.

As the time passed, the fermented milk of the powdered state which closed the fermentation and let freeze-dry could know visibility change nearly had no with the initial bacteria number. A week or greater at least could know without the difference the change of both the liquid the same experiment was enforced in the room temperature in the room temperature and powdered viable cell count compared to the things kept in refrigeration and the some extent difference had, and but big, and the archaic viable cell count to keep. Moreover, in case of the dry fermented milk, even if the water was put and it again converted into the liquid, the youghurt beverage and difference of the drying former could not be out found at taste or flavor.

▩ Effects of the Invention

As described above, in the invention, it can obtain the lactic acid bacteria count of the level which nearly thes same like the fermented milk using the existing milk and vegetable the liquid and the powdered fermented milk which it manufactures by using the soy oil and rice milk can replace the animal food with the vegetability profitabling to the health. Moreover, in this way, the manufactured vegetability liquid type fermented milk is freeze-dry let. The integrity and portability can be improved remarkably while the survival rate of the lactobacillus nearly does not change.

Scope of Claims

Claim 1 :

The method it characterizes to cultivate the mixture of the rice milk and soy oil the strain of the strain of the Bifidobacterium and Lactobacillus species and mixing and ferment and for manufacturing the youghurt beverage.

Claim 2 :

The method for characterizing to mix the soy oil and rice milk to the ratio of 1:10 to 10:1 and use as to the first claim.

Claim 3 :
The method for characterizing to mix the soy oil and rice milk to the ratio of 8:2 and use as to claim 2.

Claim 4 :
The method for characterizing the rice milk the rice milk lets be sugared the Cynanchi Radix, the brown rice, or their mixture as to the first claim and it obtains.

Claim 5 :
The method the sugar or oligosaccharide is added in the soy oil and rice milk before cultivating as to the first claim with strain and for characterizing that the final sweetness degree adjusts so that 18 brix be.

Claim 6 :
The method which is selected as to the first claim from the group consisting of the lactobacillus bulgaricus, the Lactobacillus acidophilus, and the Lactobacillus casei, and the strain of the Lactobacillus species characterizes to be selected from the group consisting of the lactobacillus bulgaricus, the Lactobacillus acidophilus, and the Lactobacillus casei.

Claim 7 :
The method the Bifidobacterium bifidum being used as to claim 6 as the strain of the Bifidobacterium, and for characterizing that the lactobacillus bulgaricus is used as the strain of the Lactobacillus species.

Claim 8 :
The method for characterizing that the Bifidobacterium bifidum and lactobacillus bulgaricus are used as to claim 7 in the form of the respective starter as the concentration of 1% for the fermentation.

Claim 9 :
The form supplied separately the Bifidobacterium bifidum and lactobacillus bulgaricus as to claim 7 the respective initial bacteria number 10.5The method for characterizing to use as the ratio of a /㎖ in the fermentation.

Claim 10 :
The method for characterizing to additionally let freeze-dry the manufactured youghurt beverage as to the first claim and manufacture the fermented milk with powdery.

Drawings

Fig. 1

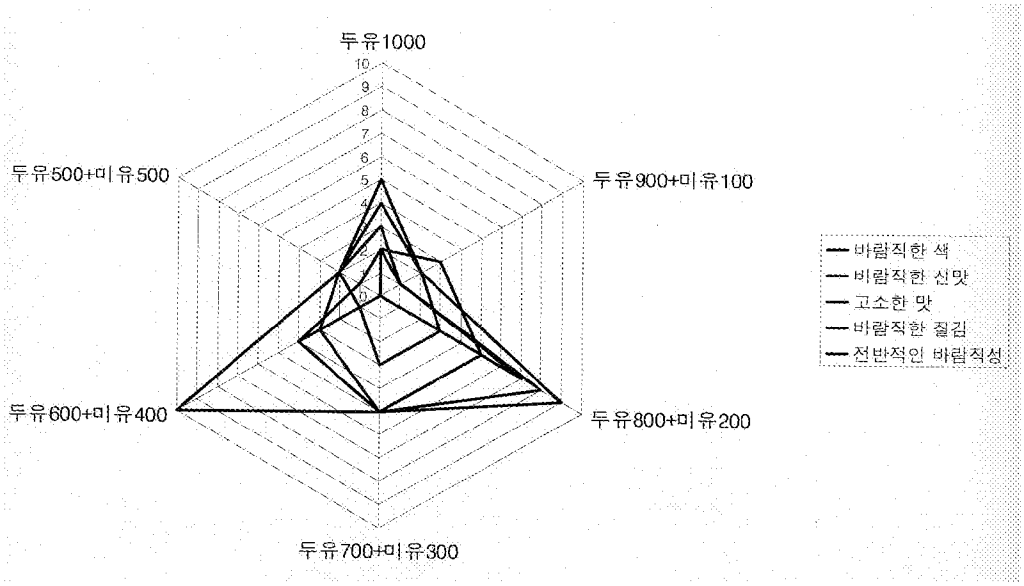

Fig. 2

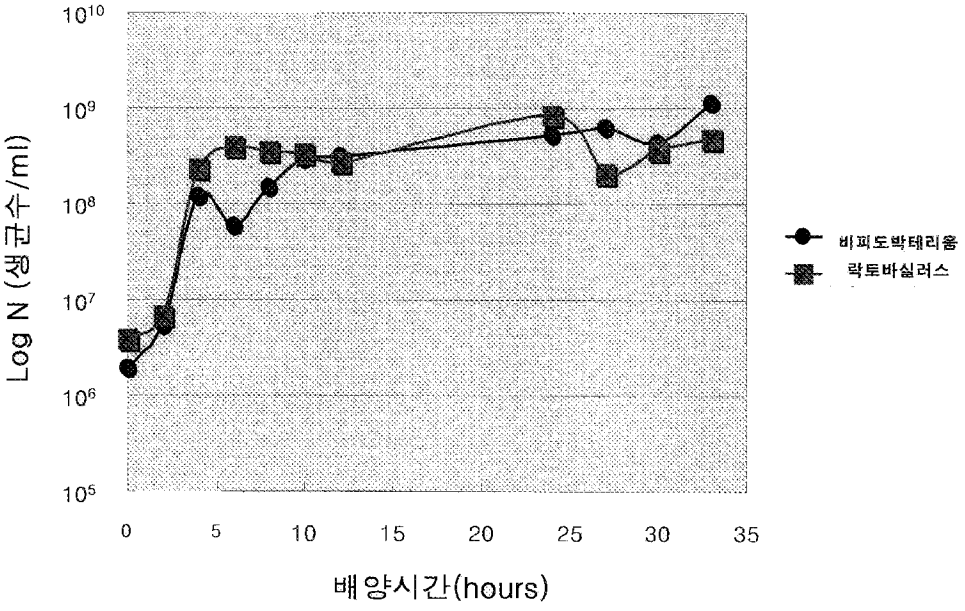

Fig. 3

DERWENT-ACC-NO: 2003-501390
DERWENT-WEEK: 200347

TITLE: Process for preparing liquid and powder types of fermented vegetable milk

INVENTOR: HONG H O ; KANG C M ; KIM W Y ; PARK H S

PATENT-ASSIGNEE:

ASSIGNEE	CODE
HONG H O	HONGI
KANG C M	KANGI
KIM W Y	KIMWI
PARK H S	PARKI

PRIORITY-DATA: 2001KR-055799 (September 11, 2001)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE
KR 2003022942 A	March 19, 2003	KO

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
KR2003022942A	September 11, 2001	2001KR-055799	

INT-CL-CURRENT:

TYPE	IPC	DATE
CIPS	A23 C 9/12	20060101

ABSTRACTED-PUB-NO: KR 2003022942 A
BASIC-ABSTRACT:

NOVELTY - Provided is a process for preparing liquid and powder types of fermented vegetable milk using legumes and rice as main ingredients to improve its preservability and distribution.

DESCRIPTION - The process for preparing liquid type of fermented vegetable milk is characterized by culturing a mixture of soy milk and rice milk with bifidobacterium and Lactobacillus sp. strains and fermenting it, wherein the mixing ratio of soy milk to rice milk is 1:10-10:1, the rice milk is obtained by saccharifying polished or unpolished rice or a mixture thereof. The powder type of fermented vegetable milk is manufacture by freeze-drying the prepared liquid type of vegetable milk to minimize the destroy of nutrients.

ABSTRACTED-PUB-NO: KR 2003022942 A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.1/10

TITLE-TERMS: PROCESS PREPARATION LIQUID POWDER TYPE FERMENTATION VEGETABLE MILK

DERWENT-CLASS: D13

CPI-CODES: D03-B; D03-B07; D03-B11;

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: 2003-133946

EXHIBIT J

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Wang et al.
Appl. No.:	10/598,909
Conf. No.:	1906
Filed:	September 14, 2006
Title:	DELIVERY OF FUNCTIONAL INGREDIENTS
Art Unit:	1655
Examiner:	Q. Mi
Docket No.:	0112701-00753

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I hereby state as follows:

1. My experience and qualifications are as follows:

I obtained B.S. degree and Master degree in Chemical Engineering from Dalian Polytechnic University (DPU), China. In 1993, received PhD in Chemistry from Swiss Federal Institute of Technology Zurich (ETH-Z). In the same year, I joined Nestlé Research Centre (NRC) in Lausanne. Since 2005, I was leading the Bioactives & Micronutrients Group, where my responsibility was to develop natural extraction technologies and concepts of bioactive delivery for healthy and tasty foods. Since July 2010, I have been appointed as the head of Nestlé Research Centre Beijing, to conduct nutrition research to promote bone&joint, cardiovascular and metabolic health.

2. I am one of the named inventors of the above-identified patent application and am therefore familiar with the inventions disclosed therein.

3. I have reviewed the outstanding Office Action dated November 16, 2010 pending against the above-identified patent application. In addition to considering the outstanding Office Action, I have reviewed the references cited therein, JP 09107880 to Osanai ("*Osanai*"), in view

of Journal of Agricultural and Food Chemistry to Edenharder et al. ("*Edenharder*"), Eur J. Nutr to Faulks et al. ("*Faulks*") and Royal Society of Chemistry to Hovari et al. ("*Hovari*") and further in view of JP 2003164261 to Imazawa et al. ("*Imazawa*"), as well as the pending claims.

4. Independent Claims 1, 12 and 14 recite, in part, a miscible primary composition comprising at least essential lipophilic and hydrophilic bioactive components of a material selected from the group consisting of whole fruit, vegetable material, plant material and combinations thereof, excluding insoluble fibers, in a carrier selected from the group consisting of milk, milk protein-containing carriers and combinations thereof. The essential lipophilic and hydrophilic bioactive components are extracted from the material by milling the material in the milk or milk protein-containing carrier and the insoluble fibers are removed by centrifuging the carrier after milling. Moreover, the miscible primary composition is stable, miscible and dispersible in an aqueous system.

5. Conventional techniques for extracting such bioactive components only extract some of the bioactive components from the fruit or plant material. Water extraction techniques, in which the bioactive components are extracted from insoluble fibers, preserve the natural image and nutritional functions of the bioactive components but are not very efficient. Solvent extraction techniques, while more efficient than water extraction, still fail to extract a substantial portion of the bioactive components from the fruit or plant material and simultaneously impair the nutritional functions of the bioactive components. Therefore, traditional water and solvent extraction techniques are only able to extract a few compounds of the fruit or plant material, leaving some other bioactive materials in the remaining material. For example, polysaccharides, polyphenols and other non-lipophilic compounds are not extracted together with the lipophilic components such as carotenoids, lipophilic vitamins and other lipids.

6. The claimed compositions are produced by processes that allow for the extraction of a greater amount of bioactive materials than with traditional water or solvent extraction techniques. The fruit or plant material is mixed in a milk or milk protein-containing medium and separated from insoluble fibers to obtain an aqueous suspension. By using a milk or milk

protein-containing carrier to extract the bioactive components from the fruit or plant material and centrifuging the milk or milk protein-containing carrier, the present claims provide compositions having bioactive components with improved miscibility, stability and bioavailability over conventional extraction techniques without the use of organic solvent residues. By using milk or milk proteins, soy-milk or milk-like proteins from plants, the claimed compositions provide a similar profile of the important nutrients like the whole fruit.

7. The inventors have surprisingly found that milling the material contained in the milk or milk protein-containing carrier allows for the formation of much smaller particles of ground plant material, allowing more efficient access by the milk or milk protein-containing carrier to both the water-soluble and oil-soluble bioactives of the plant material. Moreover, Applicants have found that the proteins in the milk or milk protein-containing carrier are significant for the increased extraction of the lipophilic and hydrophilic bioactive components from the plant material. Furthermore, centrifuging the milk or milk protein-containing carrier after milling of the fruit or plant materials removes the insoluble fibers and further provides the claimed composition as a whole to be stable, miscible and dispersible in an aqueous system.

8. *Osanai* fails to disclose or suggest a miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system as required by independent Claims 1, 12 and 14. *Osanai* discloses a beverage containing cow's milk, rapa gourd, spinach and lemon, among other ingredients. It is noteworthy that each of the embodiments of the beverage disclosed by *Osanai* at least includes approximately 22.5 grams of lemon. Moreover, lemon is an essential aspect of *Osanai's* beverage as it supplies vitamin C in an amount that is not satisfied with the remaining elements of the beverage. See *Osanai*, paragraph 12.

9. An experiment was performed to determine the impact of lemon on cow's milk as taught by *Osanai*. The experiment showed that the addition of 22.5 grams of lemon to 100 ml of milk led to a precipitation/coagulation of a large portion of the milk proteins in the milk causing an obvious lack of miscibility. See Exhibit A. Therefore, upon experimental testing to compare

Osanai's beverage against the claimed invention, it is clear that *Osanai* does not provide a miscible primary composition that is stable, miscible and dispersible in an aqueous system according to the claimed invention.

10. In sum, the inventors have surprisingly found that the milk proteins are essential for the improved extraction of the lipophilic bioactive components according to the claimed invention. The claimed miscible primary composition comprising a milk-based carrier that is stable, miscible and dispersible in an aqueous system provides the optimal conditions for extracting the most lipophilic bioactive components from plant materials. In contrast, because of the precipitation/coagulation of a large portion of the milk proteins in the beverage of *Osanai*, these precipitated or coagulated proteins are immiscible in solution and are no longer free to extract the lipophilic bioactive components of plant materials. This reduces the effectiveness of the extraction and the amount of the extracted bioactive components that could end up in the beverage. As a result, the miscible primary composition of the claimed invention is a distinguishable product over the immiscible beverage resulting from the components and process of *Osanai*.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

Date: 2011-05-06

Print Name Junkuan Wang

EXHIBIT A

Milk mixed with lemon according to the Osanai instruction:
(no other vegetables were added)

1. Lemon 22.5g mixed with 100mL cow's milk (Nestle UHT milk commercial product).
2. Add extra cow's milk (Nestle UHT milk commercial product) to adjust the sun total to 200mL.
3. Picture was taken after sample being kept quiescent for 10 minutes.
4 . Product pH is 4.26 @ 25C

Milk + lemon